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(54) Title: HIGH-AFFINITY OLIGONUCLEOTIDE LIGANDS TO SECRETORY PHOSPHOLIPASE A2(SPLA2) (57) Abstract: <p>Methods are described for the identification and preparation of high-affinity nucleic acid ligands to human secretory phospholipase A₂(sPLA₂) and human immunodeficiency virus type-1 GAG (HIV-1 GAG). Included in the invention are specific RNA ligands to sPLA₂ and HIV-1 GAG identified by the SELEX method. Also included are high-affinity modified RNA ligands and ssDNA ligands to vascular endothelial growth factor (VEGF).</p>			

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HIGH-AFFINITY OLIGONUCLEOTIDE LIGANDS TO SECRETORY PHOSPHOLIPASE A2 (sPLA₂)

5

FIELD OF THE INVENTION

Described herein are methods for identifying and preparing high-affinity nucleic acid ligands to secretory phospholipase A2 (sPLA₂) and human immunodeficiency type-
10 1 GAG (HIV-1 GAG). The method utilized herein for identifying such nucleic acid ligands is called the SELEX process, an acronym for Systematic Evolution of Ligands by EXponential enrichment. This application also describes high-affinity nucleic acid ligands to VEGF, identified through the SELEX method. Specifically disclosed herein are high-affinity nucleic acid ligands to sPLA₂, VEGF, and HIV-1 GAG. The invention
15 includes high-affinity RNA ligands that bind to sPLA₂ and inhibit the enzymatic activity of sPLA₂, modified RNA ligands and ssDNA ligands to VEGF, and RNA ligands to HIV-1 GAG. The oligonucleotides of the present invention are useful as pharmaceuticals or diagnostic agents.

20 **BACKGROUND OF THE INVENTION**

sPLA₂

Inflammation is a local response to tissue damage or foreign material which is designed to isolate and/or destroy injured tissues and foreign substances. Uncontrolled inflammatory responses may irreparably damage host tissue, as in the chronic
25 inflammation of rheumatoid arthritis or lead to multiple organ failure and death as in the systemic inflammation of septic shock .

Eicosanoids are metabolic products of the essential fatty acid, arachadonic acid and are known to play a central role in the inflammatory response. Arachadonic acid is an integral component of cell membranes where it is commonly found as an sn-2 acyl or
30 akyl ester of 3-sn-phosphoglycerides. Phospholipase A2 (PLA₂) is a class of enzymes that specifically catalyze the hydrolysis of the sn-2 acyl or alkyl ester of phosphoglycerides, producing equimolar quantities of lysophospholipids and free fatty

acids. The PLA₂ catalyzed hydrolysis of one such membrane phospholipid, alkyl-arachidonyl-glycerophosphatidylcholine, yields free arachidonic acid and lyso-platelet activating factor (lyso-PAF), the precursor of PAF, in equimolar amounts. Since the availability of free arachidonic acid is rate limiting for eicosanoid synthesis, the pro-inflammatory role of PLA₂ is thought to be the consequence of its role in arachadonic acid metabolism.

Three mammalian PLA₂ enzymes are known. Pancreatic PLA₂ is related to the type I PLA₂ from the venom of Elapidae and Hydrophide both in primary and tertiary structure. A digestive enzyme, synthesized as a proenzyme in the pancreas, it is unlikely to play a central role in inflammatory conditions.

Two non-pancreatic mammalian enzymes have been described. One is a high molecular weight intracellular enzyme. The other is a soluble enzyme, sPLA₂, and is of particular interest because it has been isolated from inflammatory exudates, such as synovial fluid (Seilhamer *et al.* (1989) J. Biol. Chem. 264:5335-5338) and from platelets (Krammer *et al.* (1989) J. Bio. Chem. 264:5768-5775). sPLA₂ is known by several equivalent names, including secretory phospholipase A₂, soluble phospholipase A₂ and synovial phospholipase A₂, all of which can be used interchangeably. This enzyme, which has been sequenced, has a molecular weight of 14 kD, a pI > 10 and is homologous to type II PLA₂ from the venoms of Crotalidae and Viperidae (Krammer *et al.* (1989) *supra*; Scott *et al.* (1991) Science 254:253-255; Wery *et al.* (1991) Nature 352:79-82).

The involvement of sPLA₂ in the inflammatory response is supported by two types of data. First, elevated levels of serum PLA₂ activity have been observed in diseases such as endotoxemia (Vadas and Hay (1983) Can. J. Physiol. Pharmacol. 61:561-566), sepsis (Vadas (1984) J. Lab. Clin. Med. 104:873-881; Nevalainen *et al.* (1992) Clin. Chem. 38:1824-1829), rheumatoid arthritis (Pruzanski *et al.* (1988) J. Rheumatol. 15:1351-1355), pancreatitis (Nevalainen *et al.* (1993) Gut 34:1133-1136) and uremia (Costello *et al.* (1990) Clin. Chem. 36:198-200) and in some studies correlate with severity and outcome. One of the best documented cases is acute pancreatitis in which sPLA₂ immuno-reactive activity, but not pancreatic PLA₂ immuno-reactive activity, is correlated with serum PLA₂ enzymatic activity. The duration of the elevated

levels is longer for the more severe necrotizing pancreatitis than the less severe oedematous form (Nevalainen *et al.* (1993) *supra*).

Second, in both animal and tissue models (Snyder *et al.* (1993) *J. Pharmacol. and Therapeutics* 266:1147-1155), the introduction of sPLA₂ results in an inflammatory like
5 response.

VEGF

Neovascularization or angiogenesis is the process in which sprouting new blood vessels are formed from the existing endothelium in response to external stimuli that signal inadequate blood supply. Angiogenesis is generally rare under normal
10 physiological conditions but frequently accompanies certain pathological conditions such as psoriasis, rheumatoid arthritis, hemangioma, and solid tumor growth and metastasis (Folkman & Klagsbrun, 1987) *Science* 235:442-447; Kim *et al.*, (1993) *Nature* 362:841-844). Several growth factors that are capable of inducing angiogenesis *in vivo* have been identified to date including basic and acidic fibroblast growth factors (aFGF, bFGF),
15 transforming growth factors α and β (TGF α , TGF β), platelet derived growth factor (PDGF), angiogenin, platelet-derived endothelial cell growth factor (PD-ECGF), interleukin-8 (IL-8), and vascular endothelial growth factor (VEGF).

VEGF was originally purified from guinea pig ascites and tumor cell cultures as a factor that increases vascular permeability (Senger, D.R. *et al.* (1983), *Science* 219:983-
20 985) and it has therefore also been referred to as vascular permeability factor (VPF). VEGF is a heat and acid-stable, disulfide-linked homodimer. Four isoforms have been described (121, 165, 189 and 206 amino acids, respectively) and are believed to be the result of alternative splicing of mRNA. Despite the presence of an identical N-terminal hydrophobic signal sequence in all molecular isoforms of VEGF, only the two lower
25 molecular weight species are efficiently secreted (Ferrara, N. *et al.* (1991) *J. Cell. Biochem.* 47:211-218). The predominant VEGF isoform in most cells and tissues is the 165 amino acid species. Although VEGF is typically glycosylated, glycosylation is only required for efficient secretion but not for activity (Yeo, T.-K. *et al.* (1991) *Biochem. Biophys. Res. Commun.* 179:1568-1575; Peretz, D. *et al.* (1992) *Biochem. Biophys. Res.*
30 *Commun.* 182:1340-1347). The amino acid sequence of VEGF is highly conserved across species and exhibits a modest but significant homology (18-20%) to PDGF A and

B (Jakeman L.B. *et al.* (1992) J. Clin. Invest. 89:244-253; Ferrara *et al.* (1992) Endocrine Rev. 13:18-32).

Unlike other angiogenic growth factors, target cell specificity of VEGF is limited to vascular endothelial cells. The biological actions of VEGF are mediated through its
5 interaction with specific cell-associated receptors which have been identified in the majority of tissues and organs (Jakeman, L.B. (1992) J. Clin. Invest. 89:244-253). Three high-affinity receptors for VEGF have been cloned to date: flt1, kdr/flk-1 and flt4 (Vaisman, N. *et al.* (1990) J. Biol. Chem. 265:19461-19466; de Vries, C. *et al.* (1992) Science 255:989-991; Galland, F. *et al.* (1993) Oncogene 8:1233-1240). These receptors
10 belong to a family of transmembrane tyrosine kinases and bind VEGF with dissociation constants between 10^{-11} M to 10^{-12} M. Recent experiments have shown that binding of VEGF to its high-affinity receptors is significantly enhanced by heparin or cell surface-associated heparin-like molecules (Gitay-Goren, H. (1992) J. Biol. Chem. 267:6093-6098).

15 In addition to promoting the growth of vascular endothelial cells and inducing vascular leakage, VEGF is also known to induce the proteolytic enzymes interstitial collagenase, urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) (Unemori E. *et al.* (1993) J. Biol. Chem. in press; Pepper, M.S. *et al.* (1992) Biochem. Biophys. Res. Commun. 181:902-). These enzymes are known to play
20 a prominent role in angiogenesis-related extracellular matrix degradation.

As a secreted and specific mitogen for endothelial cells, VEGF may be one of the major angiogenesis inducers *in vivo*. Several recent observations have supported this notion. For example, the expression of VEGF and its receptors accompanies angiogenesis associated with (i) embryonic development (Breier, G. *et al.* (1992) Development 114:521-532), (ii) hormonally-regulated reproductive cycle and (iii) tumor
25 growth (Dvorak, H.F. (1991) J. Exp. Med. 174:1275-1278; Shweiki, D. *et al.* (1992) Nature 359:843-845; Plate, K.H. *et al.* (1992) Nature 359:845-848). It is relevant to note that aggressive tumor growth is accompanied by the generation of necrotic areas where oxygen and nutrient supplies are inadequate. Oxygen deprivation (hypoxia) in tissues is
30 a known angiogenesis stimulant. Interestingly, VEGF expression was found to be the highest in tumor cells facing the necrotic areas (Shweiki, D. *et al.* (1992) *supra*; Plate,

K.H. *et al.* (1992) *supra*). It has therefore been suggested by these authors that VEGF plays a key role in hypoxia-induced angiogenesis.

Recent experiments with neutralizing monoclonal antibodies (MAbs) to VEGF have been especially meaningful for establishing that this growth factor is an important tumor angiogenesis inducer *in vivo* (Kim, K.J. *et al.* (1993) *Nature* 362:841-844). Immunocompromized (nude) mice injected with human rhabdomyosarcoma, glioblastoma or leiomyosarcoma cell lines rapidly develop tumors. Specific neutralizing MAb to VEGF were found to inhibit the growth of these tumors. The density of tumor vasculature was decreased in MAb-treated animals as compared to controls. The same MAb, on the other hand, had no effect on the growth rate of the tumor cells *in vitro* suggesting that the growth inhibition was not mediated at the cellular level and appears to be mediated by the 165-amino acid isoform of VEGF.

HIV-1 GAG

The type-1 human immunodeficiency virus (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS) (Levy (1993) *Microbiol. Rev.* 57:183-289). There are estimated to be about 1 million people infected with HIV-1 in the United States and over 13 million worldwide. In some localities over 80% of the population is infected. The average time between HIV-1 infection and death is about ten years, making the course of treatment long and expensive. There is no cure for HIV-1 infection. Five mononucleoside drugs (AZT, ddI, ddC, d4T and 3TC) have been approved for use in the United States for the treatment of HIV-1 infection. Each of these drugs inhibits the HIV-1 reverse transcriptase protein. In addition, a drug (Invirase) that inhibits the HIV-1 protease has been approved for clinical use. However, the usefulness of these drugs is limited by the rapid emergence of drug resistance mutants (Larder (1989) *Science* 246:1155-1158; Ridky and Leis (1996) *J.Biol.Chem.* 270:29621-29623). More effective treatments or a cure for HIV-1 infection would be highly desirable. As a result, other HIV-1 proteins are being explored as possible drug targets.

The HIV-1 gag (group specific antigen; also called assemblin) protein is a polyprotein composed of several HIV-1 proteins including matrix, capsid, p1, p2, p6, and nucleocapsid. Gag is myristylated on the N-terminus. Myristylation is required for localization of gag to inner surface of plasma membrane. Once gag accumulates to a

high enough concentration at the membrane it begins to multimerize. Multimerization of gag results in the formation of an immature virion that is released from the cell. After release of the virion, gag is processed into individual proteins by the HIV-1 protease leading to the formation of what is referred to as the mature virion. From electron
5 microscopy studies, gag appears to be a cylindrical -shaped protein that is 85 Å long and 34 Å wide (Nurmut *et al.* (1994) *Virology* 198:288-296). There are estimated to be about 2000 gag proteins per virus.

The HIV-1 gag protein has multiple functions in the life cycle of HIV-1 that are reflective of the functions of its individual protein components (Gorelick and Henderson
10 (1994) *In: Human retroviruses and AIDS 1994: A compilation and analysis of nucleic acid and amino acid sequences.* (Myers *et al.* eds.) Los Alamos Natl. Lab., Los Alamos, NM, pp.III-2 to III-10). Gag contains the structural proteins of HIV-1 and most of the functions of gag revolve around packaging of the viral RNA and assembly of the virus.

The matrix protein is a multifunctional protein that functions both during viral
15 assembly and after infection. During assembly, along with the capsid protein, it is thought to mediate protein/protein interactions that direct the assembly and multimerization of the "core" of HIV-1. The NMR structure of matrix and the crystal structure of Simian Immunodeficiency Virus (SIV) matrix are known. The NMR structure of HIV-1 matrix shows that it resembles that of gamma interferon (Matthews
20 (1994) *Nature* 370:666-668).

From the crystal structure of matrix, it was concluded that it forms trimers as intermediates in the assembly of HIV. Amino acids that may be involved in the interface between adjacent molecules were also identified. Matrix also has a region that forms a loop on the protein surface which is required for incorporation of the envelope protein
25 into assembling viruses (Freed and Martin (1995) *J.Virol.* 69: 1984-1989; Freed and Martin (1996) *J.Virol.* 70: 341-51). If the envelope binding function of matrix were inhibited, then viruses without envelope proteins would result and these would be noninfectious.

After infection, matrix is required for entry of viral DNA into the nucleus prior to
30 integration (Buckrinsky *et al.* (1993); *Nature* 365: 666-669). Tyrosine phosphorylation of matrix allows it to bind to the HIV-1 integrase protein/viral DNA complex (Gallay *et*

al. (1995) *Cell* 83: 569-576). The lysine-rich nuclear localization signal of matrix, located between amino acids 18 and 28, then directs localization of the integration complex into the nucleus. The vpr protein can also mediate nuclear import of integration complexes, and it has been shown that deletion of both matrix and vpr is required to
5 inhibit nuclear import. Therefore, a drug may need to inhibit the function of both vpr and matrix in order to affect nuclear import and integration.

The capsid protein ultimately forms the electron dense, cone-shaped core of HIV-1. Amino acids 240-430 are involved in dimerization of capsid. Capsid is phosphorylated on serine and threonine, but the function of this phosphorylation is
10 unknown. Capsid binds stoichiometrically to cyclophilin A, a cellular protein, leading to its incorporation into mature HIV-1 particles (Luban *et al.* (1994) *Cell* 73:1067-1078; Thali *et al.* (1994) *Nature* 372:363-365; Franke *et al.* (1994) *Nature* 372:359-362). Amino acids between positions 180-300 and capsid are required for cyclophilin binding. Cyclophilin A is the target for the action of immunosuppressive drugs related to
15 cyclosporin A. Cyclosporin A and non-immunosuppressive derivatives such as Sandoz NM811 prevent cyclophilin A incorporation into HIV-1 and severely reduce HIV infectivity (Steinkasserer *et al.* (1995) *J. Virol.* 69:814-824; Bartz *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92: 5381-5385). Mutants of capsid that do not bind cyclophilin A are also noninfectious. Thus, an inhibitor of capsid could prevent viral assembly or
20 infectivity.

The nucleocapsid protein packages HIV-1 RNA into assembling viruses. Both nucleocapsid and gag bind to a specific region called Ψ that is about 200 bases long and is located at the 5' end of the HIV-1 RNA (Aldovini and Young (1990) *J. Virol.* 64:1920-1926; Luban and Goff (1991) *J. Virol.* 65:3203-3212; Hayashi *et al.* (1992) *Virology* 188:590-599; Berkowitz *et al.* (1993) *J. virol* 67:7190-7200; Richardson *et al.*
25 (1993) *J. Virol.* 67:3997-4005; Sakuguchi *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:5219-5223). Two HIV-1 RNAs are packaged into each virus in a parallel orientation. Dimerization of the RNA is mediated by a "dimer linkage site" that is thought to be within or overlapping Ψ (Darlix *et al.* (1990) *J. Mol. Biol.* 216:689-699; Marquet *et al.*
30 (1991) *Nucl. Acid Res.* 19:153-159; Awang and Sen (1993) *Biochem.* 32:11453-11457; Skripkin *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:4945-4949). It has been suggested

that HIV-1 RNA dimerization may involve guanine quartet structures (Sundquist and Heaphy (1993) Proc. Natl. Acad. Sci. USA 90:3393-3397). However, more recently strong evidence for the involvement of kissing loops in the binding of HIV RNA to gag has been obtained (Laughrea *et al.* (1994) Biochemistry 33:13464-13474; Paillart *et al.* (1994) J. Biol. Chem. 269:27486-27493; Skripkin *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:4945-4949). The RNA sequences within the Ψ region that are required for gag binding appear to overlap with those required for packaging of viral RNA (Luban and Goff (1994) J. Virol. 68:3784-3793), but it is not clear yet if sequences required for gag binding to RNA and packaging of the RNA into the virus are identical. Nucleocapsid binding to Ψ requires two zinc finger domains and a basic region (Dorfmann *et al.* (1993) J. Virol. 67:6159-6169; Dannull *et al.* (1994) EMBO J. 13:1525-1533). The structure of nucleocapsid has been determined by nuclear magnetic resonance spectroscopy (Morellet *et al.* (1994) J. Mol. Biol. 235:287-301).

The nucleocapsid protein has nucleic acid annealing and melting activities which are thought to be required at several points in the viral life cycle such as (1) melting of the tRNA_{Lys,3} reverse transcription primer and annealing to the viral RNA, (2) melting and reannealing of viral DNA to viral RNA at various "jumping" steps of reverse transcription, and (3) melting and annealing of two genomic RNAs that are subsequently packaging together into assembling virions (Darlix, *et al.* (1995) J.Mol.Biol. 254:523-537). Disruption of events (1) or (2) by nucleic acid ligands would inhibit reverse transcription and prevent provirus formation. Disruption of event (3) would lead to viral particles that would lack viral RNA. While such particles would be "infectious" in the sense they would be able to bind and fuse to cells, they would not result in the production of new virions within those cells.

The p6 protein may play a role in budding of viruses from cells since mutants that lack p6 are defective in budding (Gottlinger *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:3195-3199). The p6 protein also binds the HIV-1 vpr protein leading to its stoichiometric incorporation into viruses (Paxton *et al.* (1993) J. Virol. 67:7229-7237). Vpr is involved in the nuclear localization of viral DNA prior to chromosomal integration. Vpr lacks a classical nuclear localization signal, so it has not been clear how it becomes localized in the nucleus. However, recently it has been reported that vpr may

bind to the glucocorticoid receptor which can translocate to the nucleus (Refaeli *et al.* (1995) Proc. Natl. Acad. Sci. USA 92:3621-3625). Vpr, along with matrix, is required for import of the integration complex. If a drug can inhibit both vpr and matrix functions in this process, then integration and production of new viruses would be prevented. Vpr
5 has also been demonstrated to influence cellular differentiation and physiology, perhaps to favor HIV latency or replication (Levy *et al.* (1993) Cell 72:541-550; Levy *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:10873-10877). Drugs that inhibit the vpr binding function of p6 would clearly be beneficial.

The peptides p1, p2 are cleaved from gag during its proteolytic processing and
10 have been proposed to regulate the proteolysis of gag (Pettit *et al.* (1994) J. Virol. 68:8017-8027; Krausslich *et al.* (1995) J. Virol. 69:3407-3419), but they may also have other functions as well. Thus, nucleic acids that interact with p1 or p2 may disrupt the proteolytic processing of gag. This would lead to "immature" virions, which could be noninfectious.

15 In addition to gag, a gag-pol polyprotein precursor is encoded by the HIV genome. The pol gene encodes the enzymes of HIV-1 such as protease, integrase, reverse transcriptase, RNase H, and integrase. Each of these proteins plays an indispensable role in the viral life cycle and inhibition of any of them would prevent HIV-1 replication. A ligand that binds to gag could inhibit the function of these proteins
20 or their assembly into virions.

Gag is also post-translationally modified. Binding of a drug to gag could inhibit the formation of these modifications. As mentioned above, the matrix protein is phosphorylated on tyrosine, and it has been proposed that this allows matrix to bind to the integration complex of HIV-1 (Gallay *et al.* (1995) Cell 83: 569-576) in order to
25 transport it into the nucleus. Blockage of this tyrosine phosphorylation might inhibit integration. Since vpr can also fulfill this function, inhibition of the phosphorylation of matrix alone may only slow the production of HIV-1 within an infected cell. Gag is also myristylated on the amino terminus of matrix. This modification is important for the localization of gag in the plasma membrane which leads to multimerization of gag and
30 assembly of the core structure of HIV-1. Blockage of myristylation would prevent viral

assembly. The capsid protein is also phosphorylated but the function of this modification is not known.

THE SELEX PROCESS

A method for the *in vitro* evolution of nucleic acid molecules with highly specific
5 binding to target molecules has been developed. This method, Systematic Evolution of
Ligands by EXponential enrichment, termed the SELEX process, is described in United
States Patent Application Serial No. 07/536,428, entitled "Systematic Evolution of
Ligands by Exponential Enrichment," now abandoned, United States Patent Application
Serial No. 07/714,131, filed June 10, 1991, entitled "Nucleic Acid Ligands," now United
10 States Patent No. 5,475,096, United States Patent Application Serial No. 07/931,473,
filed August 17, 1992, entitled "Nucleic Acid Ligands," now United States Patent No.
5,270,163 (see also PCT/US91/04078), each of which is herein specifically incorporated
by reference. Each of these applications, collectively referred to herein as the SELEX
Patent Applications, describes a fundamentally novel method for making a nucleic acid
15 ligand to any desired target molecule.

The SELEX method involves selection from a mixture of candidate
oligonucleotides and step-wise iterations of binding, partitioning and amplification, using
the same general selection scheme, to achieve virtually any desired criterion of binding
affinity and selectivity. Starting from a mixture of nucleic acids, preferably comprising a
20 segment of randomized sequence, the SELEX method includes steps of contacting the
mixture with the target under conditions favorable for binding, partitioning unbound
nucleic acids from those nucleic acids which have bound specifically to target molecules,
dissociating the nucleic acid-target complexes, amplifying the nucleic acids dissociated
from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic
25 acids, then reiterating the steps of binding, partitioning, dissociating and amplifying
through as many cycles as desired to yield highly specific, high affinity nucleic acid
ligands to the target molecule.

The basic SELEX method has been modified to achieve a number of specific
objectives. For example, United States Patent Application Serial No. 07/960,093, filed
30 October 14, 1992, entitled "Method for Selecting Nucleic Acids on the Basis of
Structure," describes the use of the SELEX process in conjunction with gel

electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. United States Patent Application Serial No. 08/123,935, filed September 17, 1993, entitled "Photoselection of Nucleic Acid Ligands" describes a SELEX-based method for selecting nucleic acid ligands containing photoreactive groups

5 capable of binding and/or photocrosslinking to and/or photoinactivating a target molecule. United States Patent Application Serial No. 08/134,028, filed October 7, 1993, entitled "High-Affinity Nucleic Acid Ligands That Discriminate Between Theophylline and Caffeine," describes a method for identifying highly specific nucleic acid ligands able to discriminate between closely related molecules, termed

10 Counter-SELEX. United States Patent Application Serial No. 08/143,564, filed October 25, 1993, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Solution SELEX," describes a SELEX-based method which achieves highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. United States Patent Application Serial No. 07/964,624, filed October 21, 1992, entitled

15 "Methods of Producing Nucleic Acid Ligands" describes methods for obtaining improved nucleic acid ligands after the SELEX process has been performed. United States Patent Application Serial No. 08/400,440, filed March 8, 1995, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Chemi-SELEX," describes methods for covalently linking a ligand to its target.

20 The SELEX method encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX-identified nucleic acid ligands containing modified

25 nucleotides are described in United States Patent Application Serial No. 08/117,991, filed September 8, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," that describes oligonucleotides containing nucleotide derivatives chemically modified at the 5- and 2'-positions of pyrimidines. United States Patent Application Serial No. 08/134,028, *supra*, describes highly specific nucleic acid ligands

30 containing one or more nucleotides modified with 2'-amino (2'-NH₂), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). United States Patent Application Serial No. 08/264,029,

filed June 22, 1994, entitled "Novel Method of Preparation of 2' Modified Pyrimidine Intramolecular Nucleophilic Displacement," describes oligonucleotides containing various 2'-modified pyrimidines.

5 The SELEX method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in United States Patent Application Serial No. 08/284,063, filed August 2, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Chimeric SELEX" and United States Patent Application Serial No. 08/234,997, filed April 28, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Blended SELEX,"
10 respectively. These applications allow the combination of the broad array of shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable properties of other molecules. Each of the above-described patent applications which describe modifications of the basic SELEX procedure are specifically incorporated by reference herein in their entirety.

15 The isolation of specific antagonists to sPLA₂ would have multiple uses. First, sPLA₂ ligands would provide a useful tool for defining the enzyme's role in inflammatory responses and in diagnosing various inflammatory conditions. Second, sPLA₂ antagonists would be useful as an anti-inflammatory therapeutic. Given the cationic nature of sPLA₂ and the specificity of high affinity oligonucleotide ligands, SELEX technology is well suited for the isolation of sPLA₂ antagonists which would not
20 cross react with pancreatic or high molecular weight PLA₂s. The present invention demonstrates the successful isolation of high-affinity oligonucleotide antagonists to sPLA₂.

The development of high affinity ligands of VEGF would be useful in the
25 treatment of angiogenesis-mediated diseases. Herein described are high affinity nucleic acid ligands of VEGF.

SELEX ligands that bind to HIV-1 proteins provide another method of "intracellular immunization" (Baltimore (1988) *Nature* 335:395-396) against HIV-1 that differs from other nucleic acid-based technologies such as antisense (Zamecnik *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:4143-4146; Chatterjee *et al.* (1992) *Science*
30 258:1485-1488; Lisiewicz *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:11209-11213).

decoys (Sullenger *et al.* (1990) Cell 63:601-608). and ribozymes (Sarver *et al.* (1990) Science 247:1222-1225; Rossi *et al.* (1992) AIDS Res. Human Retroviruses 8:183-189), which have also been shown to have potential for being effective in preventing HIV-1 replication. In previous work, RNA molecules have been evolved that bind to the HIV-1 rev, tat, reverse transcriptase, and integrase proteins. Here the SELEX method is used to derive RNA molecules that bind to the HIV-1 gag polyprotein.

Gag is an attractive target for the development of SELEX ligands for several reasons. First gag is an RNA binding protein, which facilitates the *in vitro* evolution process. Second gag is found in the cytoplasm of a cell unlike its protein components (e.g., nucleocapsid) that are only found after viral release from the cell. This feature is an attractive one with regard to achieving intracellular immunization.

BRIEF SUMMARY OF THE INVENTION

The present invention includes methods of identifying and producing nucleic acid ligands to secretory phospholipase A2 (sPLA₂) and Human Immunodeficiency Virus type-1 gag polyprotein (HIV-1 GAG) and homologous polyproteins, and the nucleic acid ligands so identified and produced. For the purpose of this application, HIV-1 GAG refers to the polyprotein or any of its component parts (i.e., matrix, capsid, p1, p2, p6, and nucleocapsid). RNA sequences are provided that are capable of binding specifically to sPLA₂ and HIV-1 GAG. Specifically included in the invention are the RNA ligand sequences shown in Tables 2, 13, 15, 18, 19, and 20. Also herein described are high-affinity nucleic acid ligands to vascular endothelial growth factor (VEGF) identified through the SELEX method. Specifically included herein are modified RNA ligands to VEGF. Such modified RNA ligands may be prepared after the identification of 2'-OH RNA ligands or by performing the SELEX process using a candidate mixture of modified RNAs. In addition, such modified RNA ligands may be prepared from previously identified modified ligands. Post-SELEX modified RNA ligands are described herein and are provided in Table 7. Also included herein are ssDNA ligands to VEGF. The evolved ssDNA ligands are shown in Table 11.

Further included in this invention are methods of identifying nucleic acid ligands and nucleic acid ligand sequences to sPLA₂ or HIV-1 GAG comprising the steps of (a)

preparing a candidate mixture of nucleic acids, (b) partitioning between members of said candidate mixture on the basis of affinity to sPLA₂ or HIV-1 GAG, and (c) amplifying the selected molecules to yield a mixture of nucleic acids enriched for nucleic acid sequences with a relatively higher affinity for binding to sPLA₂ or HIV-1 GAG.

5 More specifically, the present invention includes the RNA ligands to sPLA₂ identified according to the above-described method, including those ligands listed in Table 2. Also included are RNA ligands to sPLA₂ that are substantially homologous to any of the given ligands and that have substantially the same ability to bind sPLA₂ and antagonize sPLA₂ activity. Further included in this invention are RNA ligands to sPLA₂ that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind sPLA₂ and antagonize sPLA₂ activity.

 Additionally, the present invention includes the RNA ligands to HIV-1 GAG identified according to the above-described method, including those ligands shown in Tables 13, 15, 18, 19, and 20. Also included are RNA ligands to HIV-1 GAG that are substantially homologous to any of the given ligands and that have substantially the same ability to bind HIV-1 GAG and inhibit the function of HIV-1 GAG. Further included in this invention are nucleic acid ligands to HIV-1 GAG that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind HIV-1 GAG and inhibit the function of HIV-1 GAG.

20 The present invention also includes modified nucleotide sequences based on the RNA ligands identified herein and mixtures of the same.

 Additionally, the present invention includes modified RNA ligands to VEGF shown in Table 7. Also included herein are ssDNA ligands to VEGF as shown in Table 11. Also included are ssDNA ligands to VEGF that are substantially homologous to any of the given ligands and that have substantially the same ability to bind VEGF and inhibit the function of VEGF. Further included in this invention are ssDNA ligands to VEGF that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind VEGF and inhibit the function of VEGF.

30

DETAILED DESCRIPTION OF THE INVENTION

This application describes high-affinity nucleic acid ligands to sPLA₂, VEGF, and HIV-1 GAG, identified through the method known as the SELEX process. The SELEX process is described in U.S. Patent Application Serial No. 07/536,428, filed June 11, 1990, entitled "Systematic Evolution of Ligands by EXponential Enrichment," now abandoned, U.S. Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled "Nucleic Acid Ligands," now United States Patent No. 5,475,096, United States Patent Application Serial No. 07/931,473, filed August 17, 1992, entitled "Nucleic Acid Ligands," now United States Patent No. 5,270,163, (see also PCT/US91/04078). These applications, each specifically incorporated herein by reference, are collectively called the SELEX Patent Applications.

In its most basic form, the SELEX process may be defined by the following series of steps:

1) A candidate mixture of nucleic acids of differing sequence is prepared. The candidate mixture generally includes regions of fixed sequences (i.e., each of the members of the candidate mixture contains the same sequences in the same location) and regions of randomized sequences. The fixed sequence regions are selected either: (a) to assist in the amplification steps described below, (b) to mimic a sequence known to bind to the target, or (c) to enhance the concentration of a given structural arrangement of the nucleic acids in the candidate mixture. The randomized sequences can be totally randomized (i.e., the probability of finding a base at any position being one in four) or only partially randomized (e.g., the probability of finding a base at any location can be selected at any level between 0 and 100 percent).

2) The candidate mixture is contacted with the selected target under conditions favorable for binding between the target and members of the candidate mixture. Under these circumstances, the interaction between the target and the nucleic acids of the candidate mixture can be considered as forming nucleic acid-target pairs between the target and those nucleic acids having the strongest affinity for the target.

3) The nucleic acids with the highest affinity for the target are partitioned from those nucleic acids with a lesser affinity to the target. Because only an extremely small number of sequences (and possibly only one molecule of nucleic acid) corresponding to

the highest affinity nucleic acids exist in the candidate mixture, it is generally desirable to set the partitioning criteria so that a significant amount of the nucleic acids in the candidate mixture (approximately 5-50%) are retained during partitioning.

5 4) Those nucleic acids selected during partitioning as having the relatively higher affinity to the target are then amplified to create a new candidate mixture that is enriched in nucleic acids having a relatively higher affinity for the target.

5) By repeating the partitioning and amplifying steps above, the newly formed candidate mixture contains fewer and fewer unique sequences, and the average degree of affinity of the nucleic acids to the target will generally increase. Taken to its extreme,
10 the SELEX process will yield a candidate mixture containing one or a small number of unique nucleic acids representing those nucleic acids from the original candidate mixture having the highest affinity to the target molecule.

The SELEX Patent Applications describe and elaborate on this process in great detail. Included are targets that can be used in the process; methods for partitioning
15 nucleic acids within a candidate mixture; and methods for amplifying partitioned nucleic acids to generate an enriched candidate mixture. The SELEX Patent Applications also describe ligands obtained to a number of target species, including both protein targets where the protein is and is not a nucleic acid binding protein.

The nucleic acid ligands described herein can be complexed with a lipophilic
20 compound (e.g., cholesterol) or attached to or encapsulated in a complex comprised of lipophilic components (e.g., a liposome). The complexed nucleic acid ligands can enhance the cellular uptake of the nucleic acid ligands by a cell for delivery of the nucleic acid ligands to their intracellular target. U.S. Patent Application No. 08/434,465, filed May 4, 1995, entitled "Nucleic Acid Ligand Complexes," which is incorporated in
25 its entirety herein, describes a method for preparing a therapeutic or diagnostic complex comprised of a nucleic acid ligand and a lipophilic compound or a non-immunogenic, high molecular weight compound.

The methods described herein and the nucleic acid ligands identified by such methods are useful for both therapeutic and diagnostic purposes. Therapeutic uses
30 include the treatment or prevention of diseases or medical conditions in human patients. Diagnostic utilization may include both *in vivo* or *in vitro* diagnostic applications. The

SELEX method generally, and the specific adaptations of the SELEX method taught and claimed herein specifically, are particularly suited for diagnostic applications. SELEX identifies nucleic acid ligands that are able to bind targets with high affinity and with surprising specificity. These characteristics are, of course, the desired properties one skilled in the art would seek in a diagnostic ligand.

The nucleic acid ligands of the present invention may be routinely adapted for diagnostic purposes according to any number of techniques employed by those skilled in the art. Diagnostic agents need only be able to allow the user to identify the presence of a given target at a particular locale or concentration. Simply the ability to form binding pairs with the target may be sufficient to trigger a positive signal for diagnostic purposes. Those skilled in the art would also be able to adapt any nucleic acid ligand by procedures known in the art to incorporate a labeling tag in order to track the presence of such ligand. Such a tag could be used in a number of diagnostic procedures. The nucleic acid ligands to sPLA₂, VEGF, and HIV-1 GAG described herein may specifically be used for identification of the sPLA₂ and VEGF proteins and the HIV-1 GAG polyprotein.

The SELEX process provides high affinity ligands of a target molecule. This represents a singular achievement that is unprecedented in the field of nucleic acids research. The present invention applies the SELEX procedure to the specific targets of sPLA₂, VEGF, and HIV-1 GAG. In the Example section below, the experimental parameters used to isolate and identify the nucleic acid ligands to sPLA₂, VEGF, and HIV-1 GAG are described.

In order to produce nucleic acids desirable for use as a pharmaceutical, it is preferred that the nucleic acid ligand (1) binds to the target in a manner capable of achieving the desired effect on the target; (2) be as small as possible to obtain the desired effect; (3) be as stable as possible; and (4) be a specific ligand to the chosen target. In most situations, it is preferred that the nucleic acid ligand have the highest possible affinity to the target.

In co-pending and commonly assigned U.S. Patent Application Serial No. 07/964,624, filed October 21, 1992 ('624), methods are described for obtaining improved nucleic acid ligands after SELEX has been performed. The '624 application, entitled

"Methods of Producing Nucleic Acid Ligands." is specifically incorporated herein by reference.

5 In the present invention, the SELEX procedure was used to isolate RNAs with specific high affinity for sPLA₂ from a degenerate library containing 30 or 50 random positions (30N or 50N). This invention includes the specific RNA ligands to sPLA₂ shown in Table 2 (SEQ ID Nos.:10-122), identified by the methods described in Examples 1 and 2.

10 This invention also includes post-SELEX modified RNA ligands to VEGF having 2'NH₂ groups on the pyrimidine residues and 2'-O-methyl groups on various purine residues. In addition, nucleotides that contain phosphorothioate backbone linkages were added at the 5' and 3' ends of the ligands in order to reduce or prevent degradation by exonucleases. Internal backbone positions were also identified in which phosphorothioate linkages could be substituted, without the loss of binding affinity, to reduce or prevent endonucleolytic degradation. The post-SELEX modified RNA ligands
15 provided in Table 7 (SEQ ID NOS:126-137) demonstrate an ability to inhibit the activity of exonucleases and endonucleases, without affecting binding affinities.

Further, this invention includes ssDNA ligands to VEGF. The specific ssDNA ligands are shown in Table 11 (SEQ ID NOS:138-209).

20 In the present invention, two SELEX experiments were also performed in order to identify RNA with specific high affinity for HIV-1 GAG from degenerate libraries containing 50 random positions (50N) (Tables 12 and 14). This invention includes the specific RNA ligands to HIV-1 GAG shown in Tables 13, 15, 18, 19, and 20 (SEQ ID NOS: 229-248, 255-256, 258, 263, 265, 268-285), identified by the methods described in Examples 13 and 14. This invention further includes RNA ligands to HIV-1 GAG which
25 inhibit the function of HIV-1 GAG.

The scope of the ligands covered by this invention extends to all nucleic acid ligands of sPLA₂ and HIV-1 GAG, modified and unmodified, identified according to the SELEX procedure. The scope of the invention also extends to modified RNA ligands and all ssDNA ligands to VEGF identified according to the SELEX process. More
30 specifically, this invention includes nucleic acid sequences that are substantially homologous to the ligands shown in Tables 2, 7, 11, 13, 15, 18, 19, and 20. By

substantially homologous it is meant a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%. A review of the sequence homologies of the nucleic acid ligands shown in Table 2 for sPLA₂, Tables 7 and 11 for VEGF, and Tables 13, 15, 18, 19, and 20 for HIV-1 GAG shows that sequences with little or no primary sequence homology (e.g., 25% for HIV-1 GAG) may have substantially the same ability to bind sPLA₂, VEGF, and GAG, respectively. For these reasons, this invention also includes nucleic acid ligands that have substantially the same structure and ability to bind sPLA₂, VEGF, and HIV-1 GAG, as the nucleic acid ligands shown in Tables 2, 7, 11, 13, 15, 18, 19 and 20. Substantially the same ability to bind sPLA₂, VEGF, and HIV-1 GAG means that the affinity is within one or two orders of magnitude of the ligands herein. It is well within the skill of those of ordinary skill in the art to determine whether a given sequence--substantially homologous to those specifically described herein--has substantially the same ability to bind sPLA₂, VEGF, or HIV-1 GAG.

Inhibition of HIV-1 GAG function by the SELEX -derived ligands includes inhibition of the function of the GAG polyprotein or any of component parts. For example, inhibition of matrix or capsid interactions could inhibit viral assembly. Inhibition of matrix or of vpr incorporation by p6 could lead to viruses that would be unable to integrate and therefore unable to replicate in cells. Inhibition of cyclophilin incorporation by capsid would also be expected to lead to the production of noninfectious virus for reasons mentioned above. Inhibition of nucleocapsid function could inhibit viral RNA packaging which would lead to the formation of noninfectious virus. Inhibition of nucleocapsid could also lead to inhibition of nucleic acid annealing activities. Inhibition of p2 or p1 could lead to inhibition of proteolytic maturation of HIV.

This invention also includes the ligands as described above, wherein certain chemical modifications are made in order to increase the in vivo stability of the ligand or to enhance or mediate the delivery of the ligand. Examples of such modifications include chemical substitutions at the sugar and/ or phosphate and/or base positions of a given nucleic acid sequence. See, e.g., U.S. Patent Application Serial No. 08/117,991, filed September 9, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," specifically incorporated herein by reference. Other

modifications are known to one of ordinary skill in the art. Such modifications may be made post-SELEX (modification of previously identified modified or unmodified ligands) or by incorporation into the SELEX process.

5 As described above, because of their ability to selectively bind sPLA₂, VEGF, and HIV-1 GAG, the nucleic acid ligands to sPLA₂, VEGF, and HIV-1 GAG described herein are useful as pharmaceuticals. This invention, therefore, also includes a method for treating inflammation by administration of a nucleic acid ligand capable of binding to sPLA₂, a method for treating angiogenesis-mediated diseases by administration of a nucleic acid ligand capable of binding to VEGF, and a method for treating HIV-1 by
10 administration of a nucleic acid ligand capable of binding to HIV-1 GAG.

Therapeutic compositions of the nucleic acid ligands may be administered parenterally by injection, although other effective administration forms, such as intraarticular injection, inhalant mists, orally active formulations, transdermal iontophoresis or suppositories, are also envisioned. One preferred carrier is
15 physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. In one preferred embodiment, it is envisioned that the carrier and the ligand constitute a physiologically-compatible, slow release formulation. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other
20 pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmacologically-acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption of the ligand. Such excipients are those substances usually and customarily
25 employed to formulate dosages for parental administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or requiring reconstitution
30 immediately prior to administration. The manner of administering formulations

containing nucleic acid ligands for systemic delivery may be via subcutaneous, intramuscular, intravenous, intranasal or vaginal or rectal suppository.

The following Examples are provided to explain and illustrate the present invention and are not intended to be limiting of the invention.

5 Example 1 describes the various materials and experimental procedures used in Examples 2-8 for the evolution of nucleic acid ligands to sPLA₂. Example 2 describes the RNA ligands to sPLA₂. Example 3 describes the minimal sequences necessary for high-affinity binding to sPLA₂. Example 4 describes the specificity of RNA ligands to human sPLA₂. Example 5 describes 2'F modification of 2'OH high-affinity ligands.

10 Example 6 describes the predicted secondary structure of high affinity ligands of sPLA₂. Example 7 describes the inhibition of PLA₂-mediated contractions. Example 8 describes inhibition of the sPLA₂ enzymatic activity. Example 9 describes the experimental procedures used to generate high-affinity nucleic acid ligands to VEGF. Example 10 describes post-SELEX modifications of VEGF RNA ligands with 2'-O-methyl groups on

15 purines. Additionally, phosphorothioate backbone substitutions were made to reduce or prevent nuclease degradation without effecting binding affinity. Example 11 describes the stability of post-SELEX modified VEGF RNA ligands to *ex vivo* rat tissue degradation. Example 12 describes obtaining ssDNA ligands to VEGF. Example 13 describes the various materials and experimental procedures used in Examples 14 and 15.

20 Example 14 describes the RNA ligands to HIV-1 GAG, affinities the ligands have for HIV-1 GAG, binding of nucleic acid ligands to different sites on the HIV-1 GAG polyprotein, the frequency of matrix and nucleocapsid ligands evolved in two separate SELEX experiments, competition between HIV-1 matrix and HIV-1 nucleocapsid

25 nucleic acid ligands for binding to the HIV-1 GAG polyprotein, binding of nucleic acid ligands to nucleocapsid proteins from diverse HIV-1 strains, minimal sequence requirement for nucleic acid ligands to bind HIV-1 GAG polyprotein, predicted structural basis for recognition of HIV-1 nucleocapsid protein by nucleic acid ligands, predicted structural basis for recognition of HIV-1 matrix proteins by nucleic acid ligands, mutated HIV-1 GAG nucleic acid ligands, and truncated nucleic acid ligands that bind to the

30 HIV-1 GAG protein. Example 15 describes modified 2'-NH₂ pyrimidine RNA ligands to HIV-1 gag.

EXAMPLE 1 - EXPERIMENTAL PROCEDURES FOR 2'-OH AND 2'-NH₂ LIGANDS TO SPLA₂

This example provides general procedures followed and incorporated into Examples 2-8 for the evolution of nucleic acid ligands to SPLA₂.

5 A. *Materials*

The human SPLA₂, rabbit anti-human SPLA₂ polyclonal antibody, and enzymatic and chromogenic substrates used in these experiments were supplied by Eli Lilly & Co. hen egg-white lysozyme (6X crystallized) was provided by Dr. S.C. Gill, Jr and is commercially available. The 2' NH₂ modified CTP and UTP were prepared according to
10 Pieken *et al.* (1991) Science 253:314-317. DNA oligonucleotides were synthesized by Operon. All other reagents and chemicals were purchased from commercial sources.

B. *The SELEX Process: 2'OH RNA*

The SELEX procedure is described in detail in U.S. Patent 5,270,163 and elsewhere. The DNA template used in 2'OH RNA SELEXes contained 30 random
15 nucleotides, flanked by the N1 template 5' and 3' fixed regions (30N1(SEQ ID NO.:1), Table 1). The fixed regions include DNA primer annealing sites for PCR and cDNA synthesis as well as the consensus T7 promoter region to allow *in vitro* transcription. These single-stranded DNA molecules were converted into double-stranded transcribable templates by PCR amplification using the primers indicated in Table 1 (SEQ ID NOS.:2
20 and 3). PCR conditions were 50 mM KCl, 10 mM Tris-Cl, pH 9, 0.1% Triton X-100, 7.5 mM MgCl₂, 1 mM of each dATP, dCTP, dGTP, and dTTP, and contained 25 U/ml of Taq DNA polymerase. Transcription reactions contained 5 mM DNA template, 5 U/μl T7 RNA polymerase, 40 mM Tris-Cl (pH 8.0), 12 mM MgCl₂, 5 mM DTT, 1 mM spermidine, 0.002% Triton X-100, 4 % PEG 8000, 2 mM each of 2'OH ATP, 2'OH GTP,
25 2'OH CTP, 2'OH UTP, and 0.31 mM α-³²P 2'OH ATP. SELEX binding reactions are outlined in Table 1. For the binding reactions, RNA molecules were incubated with SPLA₂ in TBSC buffer (137 mM NaCl, 5 mM KCl, 5 mM CaCl₂, 10 mM Tris-HCl, pH 7.4) for 15 minutes at 37°C. Protein-RNA complexes were separated from unbound RNA by nitrocellulose filter partitioning and bound RNA was isolated from filters by
30 phenol/urea extraction. The RNA was reverse transcribed into cDNA by AMV reverse

transcriptase at 48°C for 60 minutes in 50 mM Tris-Cl pH (8.3). 60 mM NaCl. 6 mM Mg(OAc)₂, 10 mM DTT, 100 pmol DNA primer, 0.4 mM each of dNTPs, and 0.4 U/μl AMV RT. PCR amplification of this cDNA resulted in approximately 500 pmol double-stranded DNA, transcripts of which were used to initiate the next round of SELEX.

5 C. *The SELEX Process: 2'NH₂ RNA*

For 2'NH₂ SELEXes, DNA templates contained 50 random nucleotides flanked by N7 (50N7 SELEX (SEQ ID NO.:4)) or by N9 (50N9 SELEX (SEQ ID NO.:7)) 3' and 5' fixed regions shown in Table 1. The RNAs transcribed from these templates contained cytidine and uridine in which the 2' OH of the ribose moiety was replaced with a NH₂ group. In these SELEXes, both nitrocellulose partitioning (50N9 SELEX: rounds 6, 7, 10 and 11; 50N7 SELEX rounds 7, 8, 11 and 12) and sPLA₂ immobilized on beads via an anti-sPLA₂ polyclonal antibody (50N9 SELEX: rounds 1-5, 8 and 9; 50N7 SELEX rounds 1-6, 9 and 10) were used to separate free from bound RNA.

Polyclonal anti-sPLA₂ agarose beads were prepared from an ammonium sulfate precipitated, anion exchange purified immunoglobulin fraction of an sPLA₂ immunized rabbit. The immunoglobulins were bound to hydrazide activated agarose beads (CARBOLINK Coupling Gel, Pierce, Rockford, IL) according to the manufacturer's instructions. The resulting immunoglobulin density was estimated to be 1.2 mg/ml of gel. Coupling of sPLA₂ was accomplished by incubating 50 μl of Ab-gel with 500 μl of 2 μM sPLA₂ in TBSC for 2 hours at 37 ° C. The washed gel, which was resuspended in 500 μl of TBSC and stored at 4°C., had a calculated sPLA₂ density of 0.2 to 2 pmol/μl of gel, assuming that 1 to 10% of the Ig fraction is anti-sPLA₂ Ab and a stoichiometry of 2 molecules of sPLA₂ bound per antibody molecule.

For rounds in which immobilized sPLA₂ was used to partition unbound RNA from sPLA₂/RNA complexes, RNA was incubated with washed sPLA₂-gel in a siliconized column for 5 minutes at 37°C, as indicated in Table 1. Unbound RNA was removed by extensive washing with TBSC. Bound RNA was eluted as two fractions; the first fraction was eluted with calcium free buffer, TBS; the second fraction was eluted with free polyclonal anti-sPLA₂ Ab in TBS and was processed for use in the following round. The percentage of input RNA eluted by each step is recorded in Table 1. For rounds in which partitioning was accomplished by nitrocellulose filter binding (Table 1).

free sPLA₂ and RNA were incubated for 5 minutes at 37°C, filtered through TBSC
 prewashed nitrocellulose filters and then washed with 3 ml of TBSC.

RNA/sPLA₂ complexes absorbed to nitrocellulose filters and fractions eluted
 from immobilized sPLA₂ were heated at 90°C for 5 minutes in 1% SDS, 2% b-
 5 mercaptoethanol and extracted with phenol/chloroform. The RNAs were then processed
 as in the 2' OH SELEXes except that 2' NH₂ CTP and 2' NH₂ UTP were substituted for
 CTP and UTP in transcription reactions.

D. Nitrocellulose Filter Partitioning

The nitrocellulose filter partitioning method was used as described in SELEX
 10 Patent Applications to determine the affinity of RNA ligands for sPLA₂ and for other
 proteins. Filter discs (nitrocellulose/cellulose acetate mixed matrix, 0.45 µm pore size,
 Millipore) were placed on a vacuum manifold and washed with 5 ml of TBSC buffer
 under vacuum. Reaction mixtures, containing ³²P labeled RNA pools and sPLA₂ were
 incubated in TBSC for 5 minutes at 37°C, filtered, and then immediately washed with 5
 15 ml TBSC. The filters were air-dried and counted in a Beckman liquid scintillation
 counter without fluor.

The equilibrium dissociation constant, K_d, for an RNA pool or specific ligand that
 binds monophasically is given by the equation

$$K_d = [P_f][R_f]/[RP]$$

20

where, [R_f] = free RNA concentration

[P_f] = free Protein concentration

[RP] = concentration of RNA/protein complexes

K_d = dissociation constant

A rearrangement of this equation, in which the fraction of RNA bound at equilibrium is
 25 expressed as a function of the total concentration of the reactants, was used to calculate
 K_ds of monophasic binding curves:

$$q = (P_T + R_T + K_d - ((P_T + R_T + K_d)^2 - 4 P_T R_T)^{1/2})$$

q = fraction of RNA bound

[P_T] = total protein concentration

30

[R_T] = total RNA concentration

Many ligands and evolved RNA pools yield biphasic binding curves. Biphasic binding can be described as the binding of two affinity species that are not in equilibrium.

Biphasic binding data were evaluated with the equation

$$q = \frac{2P_1 + R_1 + K_{d1} + K_{d2} - [(P_1 + X_1 R_1 + K_{d1})^2 - 4P_1 X_1 R_1]^{1/2}}{-(P_1 + X_2 R_1 + K_{d2})^2 - 4P_1 X_2 R_1]^{1/2}},$$

where X_1 and X_2 are the mole fractions of affinity species R_1 and R_2 and K_{d1} and K_{d2} are the corresponding dissociation constants. K_d s were determined by least square fitting of the data points using the graphics program Kaleidagraph (Synergy Software, Reading, PA).

10 E. Cloning and Sequencing

During the last round of the SELEX process, PCR of cDNA was performed with primers which contain recognition sites for the restriction endonucleases HindIII and BamHI. Using these restriction sites, the DNA sequences were inserted directionally into the pUC18 vector. These recombinant plasmids were transformed into *E. coli* strain XL-1 Blue (Stratagene, La Jolla, CA). Plasmid DNA was prepared according to the alkaline hydrolysis method (Zhou *et al.* (1990) *Biotechniques* 8:172-173) and about 100 clones were sequenced with the Sequenase sequencing kit (United States Biochemical Corporation, Cleveland, OH).

20 F. Ligand Truncation

Truncation experiments were carried out to determine the minimal sequence necessary for high affinity binding of the RNA ligands to sPLA₂. For 3' boundary determination, RNA ligands were 5' end-labeled with γ -³²P-ATP using T4 polynucleotide kinase. 5' boundaries were established with 3' end-labeled ligands using α -³²P-pCp and T4 RNA ligase. After partial alkaline hydrolysis, radiolabeled RNA ligands were incubated with sPLA₂ at concentrations ranging from 0.04 nM to 28 nM and sPLA₂/RNA complexes were separated from unbound RNA by nitrocellulose partitioning. RNA truncates that bound with high affinity were identified on high-resolution denaturing polyacrylamide gels. For each radioactively labeled ligand, two types of ladders were generated to serve as markers: 1) a partial alkaline hydrolysis ladder, and 2) a partial RNase T1 digestion ladder.

EXAMPLE 2 - RNA LIGANDS TO sPLA₂

A. 2' OH SELEX

The primary objectives of the 2' OH SELEXes were 1) to generate high affinity ligands to human sPLA₂ and 2) to determine if a non-amplifiable, non-specific competitor could provide selection pressure for high affinity ligands under experimental conditions which, in the absence of competitor, did not detectably enrich for high affinity ligands. Experimental conditions for the Control, Competition, and Standard SELEX procedures are outlined in Table 1. The (Control) SELEX experiment was initiated with 240 nM sPLA₂ and a 10-fold molar excess of 2'OH RNA randomized at 30 contiguous positions, 30N1 (SEQ ID NO.:1); these conditions were maintained throughout this SELEX. A second (Competition) SELEX, in which increased stringency was imposed by a high concentration of a non-amplifiable competitor (0.5 mM tRNA), was initiated with an aliquot of the third round Control SELEX RNA. A third (Standard) SELEX, in which stringency was increased by reducing the sPLA₂ concentration to 12 nM, while maintaining a 10-fold molar excess of RNA, was started with an aliquot of the fifth round Control SELEX RNA.

The starting pool contained approximately 5×10^{14} RNA molecules (500 pmol) and bound sPLA₂ with K_d of 240 nM. After 12 rounds, the Standard and Competition SELEX pools bound sPLA₂ biphasically; the high affinity species represented about 25% of the molecules in both pools and bound with a K_d of 4 nM. No improvement was observed in the affinity of the Control SELEX RNA. These results validate the non-amplifiable competitor strategy.

The three SELEXes showed no further improvement in affinity in subsequent rounds. Twelfth and fourteenth round cDNAs from the Standard and Competition SELEXes, respectively, were PCR amplified and cloned into pUC18; 51 clones from the Standard and 34 from the Competition SELEX were sequenced. Sequences were aligned manually and are provided in Table 2. The sequences from the 2' OH SELEX from 30N1 RNA are designated by ligand names with numbers only (SEQ ID NOS.:10-59).

B. 2' NH₂ SELEX

In the 2' NH₂ SELEXes, two alternate techniques were used to partition unbound RNA from sPLA₂/RNA complexes: nitrocellulose filtration and sPLA₂ immobilized on

polyclonal anti-sPLA₂ beads as outlined in Table 1. The elution of RNA from immobilized sPLA₂/RNA complexes was based on the observation that the binding of many ligands to sPLA₂ is calcium dependent and on the premise that free polyclonal antibody can compete off bound ligands.

5 The starting pools for both the 50N7 (SEQ ID NO.:4) and 50N9 (SEQ ID NO.:7) SELEXes contained approximately 5×10^{14} RNA molecules (500 pmol) and bound sPLA₂ with K_d s of 71 and 48 nM, respectively. After 11 rounds, the 50N9 SELEX pool bound sPLA₂ biphasically; the high affinity species which represented about 67% of the molecules, bound with a K_d of 1.3 nM. Only a marginal improvement in affinity (K_d =
10 17 nM) was observed for the twelfth round 50N7 SELEX. Eleventh and twelfth round cDNAs from the 50N9 and 50N7 SELEXes, respectively, were PCR amplified with primers containing restriction sites and cloned into pUC18; 40 clones from the 50N9 and 41 from the 50N7 SELEX were sequenced. Sequences were aligned manually and are shown in Table 2. The sequences from the 50N9 RNA SELEX are designated by ligand
15 names including NN (SEQ ID NOS.:60-80) and the sequences from the 50N7 RNA SELEX are designated by ligand names including NS (SEQ ID NOS.:81-96).

C. RNA Sequences

Sequences identified by the sPLA₂ SELEX procedures described above are shown in Table 2 (SEQ ID NOS.:10-96). In the 2'OH SELEXes, 16 of 51 (Standard
20 SELEX) and 14 of 34 (Competition SELEX) sequenced ligands were unique. A unique sequence is operationally defined as one that differs from all others by three or more nucleotides. In Table 2, the RNA sequences of all 2' OH RNA ligands are shown in standard single letter code (Cornish-Bowden (1985) NAR 13:3021-3030). These clones fall into five sequence families (I-V) and a group of unrelated sequences (Orphans);
25 ligands in all six groups bind with high affinity. In addition, 12 of 38 (50N9) and 12 of 40 (50N7) sequenced ligands were unique (Table 2). All of the 50N9 ligands that bound sPLA₂ with high affinity constituted a single sequence family (VI) which is the dominant family (19 of 40) of the 50N9 SELEX. Ligand NS2 which was isolated repeatedly from the 50N7 SELEX and the 2' OH Standard SELEX orphan 60 are related to family VI.

30 The data in Table 2 define consensus sequences for families I, II, III and VI (SEQ ID NOS.:97-100). The juxtaposition of the conserved sequences of family VI to

the 5' fixed region and the conservation of the AGA by ligand NN2, suggest that these ligands require at least 3 nucleotides of the 5' fixed sequence for high affinity binding. Similarly, the family II alignment suggests that a 5' proximal CUC is necessary for high affinity binding: in some ligands this is a 5' fixed sequence, while in others it is an evolved sequence. Based on the juxtaposition of the conserved sequence of family I and II to the 3' fixed region an analogous logic predicts 3' boundaries within the 3' fixed region for ligands of these families.

The data in Table 2 also show that the distribution of sequence families I-V and orphans is different in the Standard and Competition SELEXes. Family I and V ligands occur frequently (18/51 and 9/51, respectively) in the Standard SELEX but are undetectable (0/34) in the Competition SELEX. A simple explanation for this difference is based on the observation that the high affinity binding of family I and V ligands and orphan 60 (and presumably the other orphans), unlike ligands of families II, III and IV, is calcium dependent. This correlation suggests that the free calcium was titrated by the high concentration of competitor tRNA, thus disallowing enrichment for high affinity, calcium dependent ligands.

D. Affinities

The dissociation constants for representative members of families I - VI, orphan and other ligands were determined by nitrocellulose filter binding experiments and are listed in Table 3. Unlike random RNA, all tested ligands from the 2' OH SELEXes bound biphasically. Since the affinity determinations are made under conditions of protein excess, biphasic binding suggests that the ligand exists as two affinity species that are not in equilibrium, presumably these correspond to alternatively folded conformations. In most cases, the affinity of the low affinity species resembles that of random RNA which suggests that one folded conformation binds with high affinity and that all others bind like random RNA. Most high affinity species have dissociation constants from 0.2 to 2 nM which is 1200 to 120-fold improvement over random RNA.

Unlike the 2' OH ligands, the high affinity 2' NH₂ ligands bind monophasically. The lone exception is family VI ligand NN27 (SEQ ID NO:61) which differs from the consensus sequence in having a rigorously conserved G replaced by a U. The affinity of family VI ligands is approximately 1 nM which is about a 50-fold improvement over that

of random 2' NH₂ RNA. The binding characteristics of the 2' OH and 2' NH₂ ligands demonstrate that a priori it is not possible to know if a biphasic population is a collection of biphasic binders or a mixed population of high and low affinity ligands.

EXAMPLE 3 - LIGAND TRUNCATION

5 To determine the minimal sequence necessary for high-affinity binding to sPLA₂, boundary analyses were performed on representative members of Family I (ligand 2 (SEQ ID NO.:11)), Family II (ligands 72 and 72t (SEQ ID NOS.:20 and 33)), Family V (ligands 80 and 87 (SEQ ID NOS.:53 and 54)), Family VI (ligands NN41, NN11 and NN19 (SEQ ID NOS.:68, 60, and 64, respectively). Data for truncation/boundary
10 determinations are shown in Table 2 (SEQ ID NOS.:101-121). The 3' boundaries of family I and II ligands are located in the 3' fixed region as is that of family V ligands. The position of the 5' boundary of family II ligands is in the 5' fixed region when the conserved CUC is fixed sequence. Similarly, the 5' boundary of family VI ligands is also in the 5' fixed region, while the 5' boundary of ligand 80 is the 5' G of the 5' fixed
15 region. The 3' boundary of family VI ligands coincides with the 3' end of the consensus sequence.

 Boundary locations were checked by determining the affinities of truncated ligands that approximate the minimal ligands. Although in general such truncates frequently bind as well as full length ligands, it is not a necessary outcome for at least
20 two reasons. First, the boundary is primarily defined from the difference in affinity of the boundary species and the species that is one nucleotide shorter. Second, boundary experiments examine the affinity of ligands truncated on only one end, while the minimal ligand is truncated from both ends.

 Binding affinities for full length and truncated ligands are shown in Table 3. The
25 truncates of ligands 2 and 87 (ligands 2.3 and 87.3, (SEQ ID NOS.:102 and 109) respectively) bind as well as their full length ligands, while truncates of 72, 72t and NN19 (72.c, 72t.2c and NN19.4, (SEQ ID NOS.:105, 106, and 117) exhibit a 5 to 10 fold loss in affinity. Also, the affinity of NN19.4 is restored to nearly full length levels by the addition of as few as six nucleotides to its 3' end (NN19.11, NN19.13, NN19.15 (SEQ ID
30 NOS.:118, 119, and 120). While the low affinity of truncate 87.4 (SEQ ID NO.:110)

confirms that the 5' G of ligands 80 and 87 is essential for high affinity binding. the high affinity of truncate 87.5 (SEQ ID NO:111) shows that the entire 5' fixed sequence is not necessary. The minimal ligand, operationally defined by the traditional boundary experiment, may include sequences that are required for an alternate function (i.e., proper folding; the ligands must renature in the course of the experiment) or for no function at all. In other words, ligands that are shorter than the minimal ligand may bind with high affinity.

EXAMPLE 4 - SPECIFICITY OF RNA LIGANDS TO HUMAN sPLA₂

The affinity of sPLA₂ ligands 2, 72, 80, 87, NN19 and NN19.15 (SEQ ID NOS.:11, 20, 53, 54, 64, and 117, respectively) for proteins other than sPLA₂ was determined by nitrocellulose partitioning (Tables 4 and 5). Like sPLA₂, hen egg white lysozyme, bFGF and elastase are small, highly cationic proteins. Bovine pancreatic PLA₂ is an evolutionarily and structurally related enzyme. The data in Table 4 show that the ligands are highly specific for sPLA₂. Specificity is particularly well illustrated by the affinities for bovine pancreatic PLA₂ which is 10⁴-fold less than that for human sPLA₂. These data show that in general, evolved RNA ligands to sPLA₂ bind to other proteins with an affinity similar to that of random RNA.

EXAMPLE 5 - 2'F MODIFICATION OF 2'OH HIGH AFFINITY LIGANDS

It was of interest to determine if 2'OH RNA ligands converted to a nuclease resistant form by the incorporation of 2' modified pyrimidines retained affinity for the target protein. Five 2' OH high affinity ligands to sPLA₂ (ligands 11tF, 72F, 73tF, 86tF and 87F (SEQ ID NOS.:37, 21, 32, 50 and 55, respectively) were transcribed with 2' F CTP and 2' UTP in place of CTP and UTP and their binding affinities determined. As shown in Table 3, both qualitative and quantitative changes were observed in the binding characteristics of four ligands; whereas all five bound biphasically as 2' OH RNA. they bound monophasically as 2'F RNA and their affinity was only marginally better (2 to 5 fold) than that of the random 2'F RNA control. regardless of their 2' OH affinity. On the other hand, the binding and inhibition characteristics of ligand 87 were unchanged by 2'F

modification. No degradation of this RNA was observed after incubation in 0.5X serum for 3 hours.

EXAMPLE 6 - SECONDARY STRUCTURE OF HIGH AFFINITY LIGANDS

5 In favorable instances, comparative analysis of aligned sequences enables deduction of secondary structure and structure-function relationships. Nucleotides that covary according to Watson-Crick base pairing rules are apt to be paired. Sequences that vary in composition and particularly in length are considered to be unimportant for function, while highly conserved sequences are apt to be directly involved in function.

10 Comparative analysis of family VI sequences yields a hairpin structure with a highly conserved, asymmetrical internal loop. The terminal loop (T-loop) is variable in both length and sequence (except the first and last positions) and is not apt to be directly involved in binding. The I-loop divides the stem in two. The V-stem varies in length (3-5 nucleotide pairs) and sequence. Three of the 5 base pairs are confirmed by Watson-Crick covariation. The C-stem is absolutely conserved. In this structure, the I-loop, C-
15 stem and single stranded tails are critical for binding.

The suggested structure for family III ligands is a two plane G-quartet with a closing double helix, the sequence of which is not conserved. Based on the limited data, the G-quartet loop sequences and a 5' single stranded sequence may be conserved.

20 Family II contains two very highly conserved sequences: CUUACRG and GCYGAG. Without exception, the R and Y exhibit Watson-Crick covariation which strongly suggests that they are base paired, leading to a core structure consisting of a conserved, bulged stem that has an unpaired G adjacent to the 3' end of the 3' half of the stem. This G may be a loop or a bulged nucleotide.

EXAMPLE 7 - INHIBITION OF PLA₂-MEDIATED CONTRACTIONS

25 The ligands of the invention are able to selectively block PLA₂-mediated contractions of guinea pig lung pleural strips. The procedure described by Snyder *et al.* (Journal of Pharmacology and Experimental Therapeutics (1992) 262:1147-1153) was followed to test the ability of the ligands to inhibit PLA₂-induced contraction. Ligand 19.15 (SEQ ID NO: 121) was tested in this assay and showed a dose dependent

inhibition of contraction. The same ligand was tested for its ability to inhibit an arachadonic acid-mediated contraction and did not act differently than the control. This example demonstrates that the ligands of the invention specifically inhibit PLA2-mediated contraction.

5 EXAMPLE 8 - INHIBITION OF THE sPLA₂ ENZYMATIC ACTIVITY

To directly test the ability of the RNA ligands to inhibit sPLA₂ enzymatic activity the procedure described by Reynolds *et al.* (Anal. Biochem. (1992) 204:190-197) was followed. Ligands NN11 (SEQ ID NO: 113), NN19 (SEQ ID NO: 114), NN19.11 (SEQ ID NO: 118), NN19.13 (SEQ ID NO: 119), NN19.14 (SEQ ID NO: 120), NN19.15 (SEQ ID NO: 121), 87 (SEQ ID NO: 54), 87F (SEQ ID NO: 55), 87.7 (SEQ ID NO: 112), 72t (SEQ ID NO: 104), 86t (SEQ ID NO: 49) were tested in this assay and showed an inhibitory effect.

The inhibition and affinity data for ligands 87 and 87.7 suggest a ligand with two domains. The minimal ligand, defined by boundary analysis, corresponds to the high affinity binding domain. A second domain, appended to the binding domain, is responsible for inhibition. The inhibition function may not be sequence dependent.

15 EXAMPLE 9. EXPERIMENTAL PROCEDURES FOR LIGANDS TO VEGF.

This example provides general procedures followed and incorporated into Examples 10-12 for the evolution of nucleic acid ligands to VEGF.

20 A. *Materials.*

Recombinant human VEGF (165 amino acid form; MW 46,000) was a generous gift from Dr. Napoleone Ferrara (Genentech). All other reagents and chemicals were of the highest purity available and were purchased from commercial sources.

B. *SELEX.*

25 Essential features of the SELEX protocol have been described in detail in U.S. Patent No. 5,270,163 as well as in previous papers from these laboratories (*See, e.g., Schneider et al.* (1992) J. Mol. Biol. 228:862). Briefly, DNA templates for *in vitro* transcription (that contain a region of thirty random positions flanked by constant

sequence regions) and the corresponding PCR primers were prepared chemically using established solid phase oligonucleotide synthesis protocols.

The random region was generated by utilizing an equimolar mixture of the four unmodified nucleotides during oligonucleotide synthesis. The two constant regions were designed to contain PCR primer annealing sites, primer annealing site for cDNA synthesis, T7 RNA polymerase promoter region and restriction enzyme sites that allow cloning into vectors (Table 6) (SEQ ID NOS:123-125). An initial pool of RNA molecules was prepared by *in vitro* transcription of approximately 200 picomoles (10^{14} molecules) of the double stranded DNA template utilizing T7 RNA polymerase.

Transcription mixtures consisting of 100-300 nM template, 5 units/ μ l T7 RNA polymerase, 40 mM Tris-Cl buffer (pH 8.0) containing 12 mM $MgCl_2$, 5 mM DTT, 1 mM spermidine, 0.002% Triton X-100, 4% PEG were incubated at 37°C for 2-3 hours. These conditions typically resulted in transcriptional amplification of 10 to 100-fold. Selections for high affinity RNA ligands were done by incubating bFGF with RNA for 10-20 minutes at 37°C in 50 ml of phosphate buffered saline (PBS = 10.1 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 137 mM NaCl, 2.7 mM KCl, pH 7.4) and then separating the protein-RNA complexes from the unbound species by nitrocellulose filter partitioning (Tuerk, C. and Gold, L. (1990) Science 249:505-510). The selected RNA (which typically amounted to 5-10% of the total input RNA) was then extracted from the filters and reverse transcribed into cDNA by avian myeloblastoma virus reverse transcriptase (AMV RT). Reverse transcriptions were done at 48°C (60 min) in 50 mM Tris buffer (pH 8.3), 60 mM NaCl, 6 mM $Mg(OAc)_2$, 10 mM DTT and 1 unit/ μ l AMV RT. Amplification of the cDNA by PCR under standard conditions yielded a sufficient amount of double-stranded DNA for the next round of *in vitro* transcription.

C. Nitrocellulose Filter Binding Assays.

Oligonucleotides bound to proteins can be effectively separated from the unbound species by filtration through nitrocellulose membrane filters (Yarus, M. and Berg, P. (1970) Anal. Biochem. 35:450-465; Lowary, P.T. and Uhlenbeck, O.C. (1987) Nucleic Acids Res. 15:10483-10493; Tuerk, C. and Gold, L. (1990) supra).

Nitrocellulose filters (0.2 μ m pore size, Schleicher and Schuell, Keene, NH) were secured on a filter manifold and washed with 4-10 ml of buffer. Following incubations

of ^{32}P labeled RNA with serial dilutions of the protein for 10 min at 37°C in buffer (PBS) containing 0.01% human serum albumin (HSA), the solutions were applied to the filters under gentle vacuum in 45 ml aliquots and washed with 5 ml of PBS. The filters were then dried under an infrared lamp and counted in a scintillation counter.

5 D. *Equilibrium Dissociation Constants.*

In the simplest case, equilibrium binding of RNA (R) to VEGF (P) can be described by eq. 1,

Kd



10 where $K_d = ([\text{R}][\text{P}]/[\text{R.P}])$ is the equilibrium dissociation constant. Using the mass-balance equations, the fraction of bound RNA at equilibrium (q) can be expressed in terms of measurable quantities (eq. 2),

$$q = (f/2R_t) \{ P_t + R_t + K_d - [(P_t + R_t + K_d)^2 - 4P_t R_t]^{1/2} \} \quad (2)$$

15 where P_t and R_t are total protein and total RNA concentrations and f reflects the efficiency of retention of the protein-RNA complexes on nitrocellulose filters. The average value of f for VEGF in our assays was 0.7.

Most RNA ligands exhibited biphasic binding to VEGF. For those ligands, binding of RNA to VEGF is described by a model where total RNA is assumed to be partitioned between two non-interconverting components (R_1 and R_2) that bind to VEGF with different affinities (eqs 3 and 4).

Kd1



Kd2



25 In this case, the fraction of total bound RNA (q) is given by eq. 5.

$$q = \frac{(f/2R_t) \{ 2P_t + R_t + K_{d1} + K_{d2} - [(P_t + \chi_1 R_t + K_{d1})^2 - 4P_t \chi_1 R_t]^{1/2} - [(P_t + \chi_2 R_t + K_{d2})^2 - 4P_t \chi_2 R_t]^{1/2} \}}{2} \quad (5)$$

where χ_1 and $\chi_2 (= 1 - \chi_1)$ are the mole fractions of R1 and R2 and K_{d1} and K_{d2} are the corresponding dissociation constants.

5 Internally-labeled RNA ligands used for binding studies were prepared by *in vitro* transcription using T7 RNA polymerase (Milligan *et al.* (1987) Nucl. Acids Res. 15:8783) and were purified on denaturing polyacrylamide gels to ensure size homogeneity. All RNA ligands were diluted to about 1 nM in PBS, denatured at 90°C for 2 minutes, and then cooled on ice prior to incubation with the protein. This
10 denaturation/renaturation cycle performed at high dilution is necessary to ensure that the RNA is essentially free from dimers and other higher order aggregates. Concentrations of the stock solutions of VEGF, from which other dilutions were made, were determined from the absorbance reading at 280 nm using the calculated value for ϵ_{280} of 46,600 M⁻¹ cm⁻¹ for the VEGF dimer (Gill *et al.* (1989) Anal. Biochem. 182:319). Data sets that
15 define the binding curves were fit to either eq. 2 or eq. 5 by the non-linear least squares method using the software package Kaleidagraph (Synergy Software, Reading, PA).

E. Information Boundary Determinations.

High-affinity VEGF ligands were radiolabeled at the 5'-end with γ -³²P-ATP (New England Biolabs, Beverly, MA) and T4 polynucleotide kinase (New England Biolabs,
20 Beverly, MA) for the 3'-boundary determinations, or at the 3'-end with α -³²pCp and T4 RNA ligase (New England Biolabs) for the 5'-boundary determination. Radiolabeled RNA ligands were subjected to partial alkaline hydrolysis and then selectively bound in solution to VEGF at 5, 0.5, or 0.125 nM before being passed through nitrocellulose filters. Retained oligonucleotides were resolved on 8% denaturing polyacrylamide gels.
25 In each experiment, the smallest radiolabeled oligonucleotide bound by VEGF at the lowest protein concentration defines the information boundary. Partial digests of the 5'- or the 3'-labeled RNA ligands with RNase T₁ (Boehringer Mannheim Biochemicals, Indianapolis, IN) were used to mark the positions of labeled oligonucleotides ending with a guanosine.

F. *Cloning and Sequencing.*

Individual members of the enriched pool were cloned into pUC18 vector and sequenced as described (Schneider, D. *et al.* (1992) J. Mol. Biol. 228:862-869).

G. *Receptor Binding.*

5 VEGF was radioiodinated by the Iodogen method (Jakeman *et al.* (1992) J. Clin. Invest. 89:244) to a specific activity of 2.4×10^4 cpm/ng. Human umbilical vein endothelial cells (HUVECs) were plated in 24-well plates at a density of $1-2 \times 10^5$ cells/well and grown to confluence in EGM (Clonetics, San Diego, CA) media (24-48 hrs). At confluence, the cells were washed 3 times with PBS and incubated for 2 hrs at
10 4°C in α -MEM serum-free media containing ^{125}I -labeled VEGF with or without unlabeled competitor (VEGF, EGF, or RNA). For experiments done with RNA, 0.2 units of placental RNase inhibitor (Promega, Madison, WI) were included in the media. It was determined that the RNA ligands were not degraded during the course of the experiment. At the end of the 2 hr incubation period, the supernatant was removed and
15 the wells washed 2 times with PBS. HUVECs were then lysed with 1% triton X-100/1 M NaOH and the amount of cell-associated ^{125}I -VEGF determined by gamma counting.

EXAMPLE 10 - POST SELEX MODIFICATIONS OF VEGF RNA LIGANDS.

VEGF RNA ligands are described in WO 95/07364 (PCT/US94/10306), which is incorporated herein by reference in its entirety. In an attempt to further stabilize the
20 nucleic acid ligands of the ligands described in WO 95/07364, certain post-SELEX modifications were done. The ligand NX107 (SEQ ID NO:126) was chosen as a model for post-SELEX modification. NX107 is a truncated version of Ligand 24A, which is described in WO 95/07364. All of the pyrimidines in NX107 have an NH_2 group substituted at the 2'-position of the ribose. This example describes substitution of
25 O-Methyl groups at the 2'-position of the ribose of certain of the purines of NX107. Additionally, phosphorothioate nucleotides were added at the 5' and 3' ends of the ligands and in at least one instance, at an internal position. The various substitutions to the ligand were designed to inhibit the activity of exonucleases and endonucleases, but not affect binding affinity.

To this end, certain ligands were synthesized and tested for binding affinity. The sequences and the results of the binding studies are provided in Table 7. The binding studies were performed using the protocols described in Example 9.

EXAMPLE 11 - STABILITY OF POST-SELEX MODIFIED VEGF LIGANDS TO EX
VIVO RAT TISSUE DEGRADATION.

In order to be able to quickly assess the effects of ligand modifications on stability to tissue nucleases, the following assay was developed. Brain, kidney, liver and spleen tissues were removed from a freshly sacrificed rat, washed in saline to remove blood, and sliced into approximately 10 mm³ pieces. Each piece was put into an Eppendorf tube with 50 μ l PBS and quick frozen on dry ice. Tissues from the same rat were used for all the experiments described here. The ligand to be tested was 5'-end-labeled with ³²P, added to the thawed tissue slice in 80 μ l PBS, and incubated at 37°C. Aliquots were withdrawn at 3, 10, 30, and 60 minutes, added to an equal volume of formamide dyes on ice, and quick-frozen on dry ice. The samples were run on a 20% denaturing acrylamide gel along with equal counts of the unincubated ligand, and a partial alkaline hydrolysate of the ligand (or a related ligand) for sequence markers. The gels were dried and exposed to X-ray film and a phosphorimager plate (for quantitation of degradation).

The VEGF ligands used in this study are shown in Table 7. Each ligand has the same core 24-mer sequence derived from a truncated 2'NH₂-pyrimidine SELEXed ligand (NX-107)(SEQ ID NO:126). NX-178 (SEQ ID NO:128) is the same 2' amino pyrimidine ligand with phosphorothioate backbone linked thymidine caps at the 5'- and 3'- ends of the ligand. NX-190 (SEQ ID NO:129) is an all DNA version of the same sequence with the above-described caps, and NX-191 (SEQ ID NO:130) is an all 2'OMe version. NX-213 (SEQ ID NO:131) is the capped amino ligand with all the purines 2'OMe substituted except four. NX-215 (SEQ ID NO:133) is the same as NX-213 with an internal phosphorothioate linkage between A7 and U8.

Tables 8 and 9 provide the results obtained by this assay on rat brain and kidney tissues as indicated by the percent of full length material found at the various time points. For this analysis, a ligand is still considered functionally intact with cuts in the

phosphorothioate caps. The other tissues assayed had similar results. The post-SELEX modifications were successful in protecting the ligand from various endo- and exonucleases.

EXAMPLE 12 - SSDNA LIGANDS TO VEGF.

5 This example demonstrates the ability to obtain ssDNA ligands to vascular endothelial growth factor (VEGF).

 Most of the materials and methods are the same as those described in Example 9. Two libraries of synthetic DNA oligonucleotides containing 40 random nucleotides flanked by invariant primer annealing sites were amplified by the Polymerase Chain
10 Reaction (PCR) using oligonucleotide primers as shown in Table 10 (SEQ ID NOS:216-221). The protocols for the SELEX procedure are as described by Jellinek *et al.* (PNAS (1993) 90:11227-11231), in the SELEX Patent Applications and in Example 9. VEGF protein binding assays, receptor binding assays, and information boundary determinations are also described in Example 9.

15 The ssDNA ligands identified are shown in Table 11 (SEQ ID NOS:138-199). Only the sequence of the evolved region is provided in Table 11, however, each of the clones also includes the fixed regions of either SEQ ID NO:216 or SEQ ID NO:219. Clones named with numbers only include the fixed regions of SEQ ID NO:216 and clones named with b and number included the fixed regions of SEQ ID NO:219.
20 Truncations (information boundary determinations) were performed on a number of ligands, which is also provided in Table 11 (SEQ ID NOS:200-209). Four sequence families were obtained from the alignment of the primary sequences of these ligands and a consensus sequence generated for each family (SEQ ID NOS:210-215). Orphan sequences were also identified. Select ligands were tested in the VEGF protein binding
25 assay with results being shown in Table 11. The starting DNA random pool had a binding affinity K_d of approximately 200 nM. In the VEGF receptor binding assay, the truncated clone 33t (SEQ ID NO:203) had a K_i of 3 nM.

EXAMPLE 13. EXPERIMENTAL PROCEDURES FOR LIGANDS TO HIV-1 GAG

This example provides general procedures followed and incorporated into Examples 14-15 for the evolution of nucleic acid ligands to HIV-1 GAG.

A. *Materials.*

5 The HIV-1 p55 (gag), p7 (nucleocapsid), and p15 (p7 nucleocapsid-p6) proteins from HIV-1 strain LAI (generous gifts from Drs. Tristram Parslow and Jared Clever (Dept. of Pathology and the Dept. of Microbiology and Immunology, University of California, San Francisco, CA 94143-0506)) were expressed in *E. coli* as fusions to glutathione S-transferase (GST) and purified as described (Clever *et al.* (1995) *J. Virol.* 10 69:2101-2109). They are referred to in the Examples as GST-gag, GST-p7 and GST-p15, respectively. The GST protein itself was also purified using the same procedures. The HIV-1 p24 capsid and HIV-1 p7_{MN} proteins were obtained from the NIH AIDS Research and Reference Reagent Program. The HIV-1 p17 matrix protein was 15 obtained from the MRC AIDS Reagent Project. All oligonucleotides used were synthesized by Operon, Inc. (Alameda, CA). Restriction enzymes were purchased from New England Biolabs (Beverly, MA). All other reagents, chemicals and plasmids were from commercial sources.

B. *SELEX ligand generation.*

SELEX ligands that bind to the HIV-1 gag polypeptide were derived in two 20 SELEX experiments ("A" and "B") essentially as described in U.S. Patent No. 5,270,163 (see also Tuerk and Gold (1990) *Science* 249:505-510; Tuerk *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Tuerk and MacDougall-Waugh (1993) *Gene* 137:33-39; Tuerk *et al.* (1994) in The Polymerase Chain Reaction, eds. Ferre *et al.* (Birkhauser, Springer-Verlag, New York; Jenson *et al.* (1994) *J. Mol. Biol.* 235:237-247) with the 25 following modifications. SEQ ID NO.222 was used as the starting template and SEQ ID NOS: 223 and 224 were used for the PCR primers for SELEX "A" and SEQ ID NO. 249 was used as the starting template and SEQ ID NOS. 250 and 251 were used as the PCR primers for SELEX "B." SELEX experiment "A" used a starting RNA pool of 8.4×10^{14} molecules with 50 random bases (Table 12; SEQ ID NO: 225), a binding buffer that 30 consisted of 50 mM Tris, pH 7.5, 200 mM KOAc, 5 mM MgCl₂, 1 mM dithiothreitol, and avian myeloblastosis virus (Life Sciences, Inc., St. Petersburg, FL) as the reverse

transcriptase. SELEX experiment "B" used a starting RNA pool of 7×10^{14} molecules with 50 random bases (Table 14; SEQ ID NO: 252), a gag binding buffer that consisted of 50 mM Tris, pH 7.4, 140 mM KCl, 5 mM NaCl, 5 mM $MgCl_2$, 1mM dithiothreitol (gag binding buffer) and murine leukemia virus reverse transcriptase (Superscript, Gibco, Inc., Gaithersburg, MD) as the reverse transcriptase. SELEX experiment "B" also utilized increasing concentrations of heparin (molecular weight 5,000, Calbiochem, Inc., San Diego, CA) at a 10-200 fold excess over the RNA pool in each round of SELEX to compete with nonspecifically binding RNAs in the pool for binding to gag. Both the "A" and the "B" SELEX used Taq polymerase (Perkin-Elmer Cetus, Inc., Norwalk, CT) to amplify cDNA products. Binding of RNA pools was for 5 minutes in SELEX "A" and for 10-15 minutes in SELEX "B." T7 RNA polymerase and 2'-hydroxyl nucleosides were used to transcribe the amplified cDNAs. Nitrocellulose partitioning was used for both SELEX experiments except for round 5 in SELEX "B." Column SELEX was used for round 5 in SELEX "B" as described *infra*.

C. *Subcloning of ligands.*

The ligand pool from the appropriate round was amplified using PCR primers (Table 12; SEQ ID NOS: 226 and 227) for SELEX "A," and Table 14, (SEQ ID NOS: 253 and 254) for SELEX "B" that have restriction enzyme recognition sites on the 5' and 3' ends. The PCR products were digested with the appropriate restriction enzyme (*HindIII* and *BamHI* for SELEX "A" and *BamHI* and *EcoRI* for SELEX "B") and then subcloned into pUC9 (BRL, Inc., Gaithersburg, MD) that was also digested with the appropriate restriction enzymes. The ligation was transformed into *E.coli* strain DH α .

D. *Sequence analysis of clones.*

Plasmids were prepared from the transformants using either the boiling method or the alkaline lysis method using art known techniques (see Molecular Cloning: A Laboratory Manual (Eds. Sambrook *et al.* (1989)). The plasmids were denatured with 0.5 M NaOH, desalted on a 0.5 ml G-50 Sephadex spin column, and then used for sequencing. Sequences were determined using the Sequenase version 2.0 enzyme (U. S. Biochemical, Inc., Cleveland, OH) according to manufacturer's instructions. A DNA primer labeled with phosphorous-32 at the 5'-end and corresponding to the T7 promoter (Table 12; SEQ ID NO: 228) was used as the sequencing primer.

E. *Binding affinities of ribonucleic acid ligands for target HIV-1 proteins.*

The binding affinities of ribonucleic acid ligands for the various proteins (i.e., GST-gag, GST-p7, matrix, capsid, GST, GST-p15) were measured by filtration essentially as described (Tuerk and Gold (1990) *Science* 249:505-510; Jellinek *et al.* (1994) *Biochemistry* 33:10450-10456). Briefly, radiolabeled RNA was transcribed from a PCR-generated template using T7 RNA and polymerase and α -³²P-ATP (DuPont NEN Research Products, Boston, MA). The RNA (~200 fmol; ~10,000 cpm) was mixed with the protein at concentrations ranging from 10⁻¹² to 10⁻⁶ M at 37 °C. for 5-10 minutes in a 30 μ l reaction. When the protein used was GST-p7, GST-p15 or GST-gag, a 10,000 fold molar excess of yeast tRNA was added to the RNA prior to mixing with the protein. The buffer used for binding is as described above. The reactions were vacuum-filtered through nitrocellulose filters (HAWP, Millipore, Inc. Bedford, MA). A control with no protein added was also included for each RNA to correct for the amount of the RNA that binds to the nitrocellulose filter instead of protein. The amount of labeled RNA specifically retained on the filter by protein was determined, and the apparent K_d of the protein for the RNA was obtained by plotting the amount of RNA specifically bound to protein vs. the concentration of the protein using Kaleidograph computer software (Synergy, Inc., Reading, PA).

As a positive control for binding to gag, an RNA referred to herein as Ψ -456 was used (see Example 13). Ψ -456 contains the HIV-1 RNA packaging signal (Ψ) which binds specifically to the HIV-1 gag and nucleocapsid proteins during the course of viral assembly.

The Ψ -456 RNA was made by transcription of Xho I-digested pBsP (Luban and Goff (1991) *J. Virol.* 65: 3203-3212) using T7 RNA polymerase. Ψ -456 RNA consists of 441 bases from the HIV-1_{HXB2R} RNA (containing nucleotide positions 712-1152 of HIV-1_{HXB2R} RNA; (Myers *et al.* eds. (1993) *Human retroviruses and AIDS 1993: A compilation and analysis of nucleic acid and amino acid sequences*, Los Alamos Natl. Lab., Los Alamos, NM) plus 15 bases of vector-derived sequence at the 5' end. Ψ -70 (containing nucleotide positions 712-765 of HIV-1_{HXB2R} RNA) was transcribed from a double-stranded DNA template generated by a polymerase chain reaction using pBsP as a template. Ψ -93 (containing nucleotide positions 712-788 of HIV-1_{HXB2R} RNA) was

transcribed from Nde I-digested pBsP. Ψ -136 (containing nucleotide positions 712-831 of HIV-1_{HXB2R} RNA) was transcribed from Cla I-digested pBsP.

F. Competition binding reactions.

Nucleic acid ligands (SW8.27, SEQ ID NO:236; SW8.4, SEQ ID NO:231; SW10.12, SEQ ID NO:242; SW8.24, SEQ ID NO:234) were transcribed with T7 RNA polymerase as described above using α -³²P-ATP. Each radiolabeled ligand was mixed with various amounts of unlabeled competitor RNAs (SW8.27, SEQ ID NO:236; SW8.4, SEQ ID NO:231; Ψ -456, or the starting RNA pool for SELEX "A", SEQ ID NO:225) ranging in concentration from 10⁻¹² to 10⁻⁶ M. The mixed RNAs were then added to the appropriate HIV-1 protein at a concentration of protein equal to the affinity (apparent K_d) of the protein for the radiolabeled RNA ligand. The reaction was incubated in gag binding buffer and then filtered as described above.

G. Ligand boundary determinations.

The boundaries of nucleic acid ligands were determined essentially as described by Jellinek *et al.* (1994) Biochemistry 33:10450-10456 with the following modifications. The concentration of the appropriate HIV-1 protein that was used was equal to the apparent K_d. The HIV-1 p17 matrix protein was used to determine the boundaries of matrix nucleic acid ligands and the GST HIV-1 p15 (nucleocapsid-p6) protein was used to determine the boundaries of nucleocapsid nucleic acid ligands. In some binding reactions, especially with high affinity ligands, a 10,000 fold molar excess of heparin or yeast tRNA over the ligand was added to suppress nonspecific binding to short RNA fragments. For the RNase T₁ digestion of ligands, yeast tRNA was added at a concentration of 0.3 mM and then incubated for 5 minutes at 37 °C.

H. Truncated and mutated nucleic acid ligands.

Truncated nucleic acid ligands were generated by one of two methods. In one method, an oligonucleotide consisting of the antisense strand of the T7 ϕ 10 promoter plus the antisense sequence of the desired truncated ligand was synthesized. This was hybridized to an oligonucleotide with the sequence corresponding to the sense strand of the T7 ϕ 10 promoter. Such complexes can be used as a template for transcription by T7 RNA polymerase to generate truncated nucleic acid ligands (Milligan *et al.* (1987) Nuc. Acids Res. 15:8783-98; Milligan and Uhlenbeck (1989) Methods Enzymol. 180:51-62).

In another method, two oligonucleotides were synthesized. The sequence of one oligonucleotide contained the sense sequence of the T7 RNA polymerase $\phi 10$ promoter and part of the 5' end of the truncated ligand. The sequence of the second oligonucleotide encoded the antisense sequence of the 3' end of the truncated ligand. The oligonucleotides were designed so that they overlapped. These oligonucleotides were subjected to 3 rounds of the polymerase chain reaction in order to generate a double-stranded T7 RNA polymerase transcription template.

Mutated nucleic acid ligands were generated in a similar manner by incorporating the desired base changes into the oligonucleotides used to generate the transcription templates.

EXAMPLE 14. RNA LIGANDS TO HIV-1 GAG

A. In vitro evolution of RNAs that bind to gag.

A GST-gag fusion protein was used in both SELEX experiments. GST alone has a low affinity ($K_d > 10 \mu\text{M}$) for random RNA, whereas GST-gag has a higher affinity ($K_d = 0.1-1 \mu\text{M}$) for random RNA. Therefore, as a result of the properties of the SELEX method, the majority of the evolved ligands bind to gag and not GST.

SELEX "A" was carried out for a total of 11 rounds. The sequence of the RNA pool was determined at rounds 5, 6, and 7 and found to be nonrandom. The K_d of the round 10 pool was determined and found to be 1.5 nM. In the same experiment, the K_d of Ψ -456, the HIV-1 gag-binding RNA element, was 1.1 nM. On the basis of the K_d and the nonrandomness of the sequences, the ligand pools from rounds 8 and 10 were cloned into pUC9 for sequence analysis of individual ligands.

Sequences were obtained from 34 clones, 19 from round 8 and 15 from round 10. Twelve of the sequences were identical and are represented by ligand SW8.4 (SEQ ID NO: 231). One ligand, represented by ligand SW10.22 (SEQ ID NO: 243), was almost identical to the ligand SW8.4 family. Two other sequences were identical to ligand SW8.6 (SEQ ID NO: 232). Another ligand, SW8.27 (SEQ ID NO: 236) differs from SW8.6 by two bases. Ligands SW8.24 (SEQ ID NO: 234) and SW10.39 (SEQ ID NO: 247) differ by one base. In summary, from 34 clones, 23 (SEQ ID NOS: 229-248) unique sequences were obtained.

The 23 unique ligands were screened for binding to the GST gag protein at 0.0nM, 0.1 nM and 10.0 nM gag, concentrations that bracket the average K_d of the round 10 pool. Round 0 and Ψ-456 RNA were added as negative and positive controls, respectively, for binding to gag. All of the ligands were also filtered in the absence of GST-gag (0.0nM) to detect nitrocellulose binding sequences. The binding was done in the presence of a 10,000 fold molar excess of yeast tRNA. Three ligands were found to be nitrocellulose binders. The data for the 20 ligands that bound to GST-gag are summarized in Table 16. Several ligands bound well to GST-gag, especially the most frequent ligand SW 8.4 (and the related ligand SW 10.22), SW 8.1, SW 8.27, and SW 10.28.

It has been reported previously that ligands which bind to nitrocellulose filters are rich in guanine. The gag ligands from SELEX "A" that bound to nitrocellulose had 18 or more guanines in the randomized region, whereas the gag ligands that bound to gag had 14 or less guanines in the randomized region. The sequences of 20 clones from rounds 8 and 10 of SELEX "A" that bound to the gag protein are shown in Table 13.

SELEX "B" was carried out for a total of 9 rounds. After 4 rounds of SELEX, the amount of RNA that was binding to nitrocellulose filters had risen. Therefore, we employed an alternate partitioning strategy for round 5. Round 5 was performed using "column SELEX." 0 or 10 nmoles GST-gag protein was bound to 50 ml glutathione Sepharose 4B (Pharmacia) on ice for 15 minutes. 150 nmoles of round 5 RNA pool was added and incubated at 37° C. for 15 minutes. The beads were pelleted in a microfuge at 1000 rpm for 2 minutes. The aqueous supernatant was saved. The pelleted beads were washed in 1 ml of ice cold gag binding buffer and then the beads were immediately repelleted. The beads were washed in such a manner for a total of 6 times. All of the washes and the beads were Cherenkov-counted. At this point, the beads incubated with GST-gag bound 180 fmoles RNA, while the beads with no GST-gag prebound had bound 3.3 fmoles RNA. The beads were then washed with 2-250 µl aliquots of gag binding buffer containing 5 mM reduced glutathione to elute GST-gag RNA complexes. The eluted GST-gag RNA complexes were phenol extracted, ethanol precipitated, and the recovered RNA was reverse-transcribed, amplified by PCR, and transcribed (as described in Tuerk and Gold (1990) Science 249:505-510) in preparation for the round 6

selection. The enrichment of ligands in this round was calculated to be as much as 830 fold, substantially more than that for other rounds in which a standard nitrocellulose partitioning was used (average of about 100 fold enrichment).

5 The sequence of the RNA pools in SELEX B were determined at rounds 0, 2, 4, 6, and 8. The sequences of rounds 0, 2, and 4 were random. The sequences of the RNA pool from rounds 6 and 8 were nonrandom. The high degree of enrichment that may have been achieved in round 5 using column SELEX may explain the sudden shift in the sequence of the RNA pools from random in round 4 to nonrandom in round 6.

10 The K_d of the round 0, 4, and 8 pools was determined and found to be $\sim 1 \mu\text{M}$, 8.5 nM, and 1.1 nM, respectively. In the same experiment, the K_d of Ψ -456, the HIV-1 gag-binding RNA element, was 5.2 nM.

15 The ability of Ψ -456, round 4, and round 8 RNA pools from SELEX "B" to compete with Ψ -456 for binding to gag was examined. The concentration of ^{32}P -labeled Ψ -456, round 4, and round 8 RNA pools was approximately 150 pM, the concentration of GST-gag was fixed at approximately 1 nM, and the concentration of unlabeled Ψ -456 was varied. The amount of unlabeled Ψ -456 required to compete off 50% of the ^{32}P -labeled Ψ -456, round 4, or round 8 RNA pools was a 20, 74 or 630 fold excess, respectively. Therefore, SELEX ligands can compete for binding to gag more effectively than Ψ -456. Since SELEX-derived gag ligands have a lower K_d for gag than Ψ -456 and since they compete more effectively for binding than does Ψ -456, in a gene therapy setting SELEX-derived gag ligands may not need to be expressed to a level comparable to the HIV-1 RNA in order to effectively compete with Ψ for binding to gag.

20 On the basis of the K_d and the nonrandomness of the sequence pools after round 6, the ligand pool from rounds 6 and 8 of SELEX "B" were subcloned into pUC9 for sequence analysis of individual ligands.

25 Nine unique sequences from round 6 and 13 unique sequences from round 8 of SELEX "B" were found (Tables 15 and 18). Seven clones were identical to ligand ML8.7 (SEQ ID NO: 255) and two more, represented by ligand ML8.14 (SEQ ID NO: 258) were similar to ligand ML8.7. The ligands from SELEX "B" were also screened for binding to GST-gag or nitrocellulose filters as described above. The results are shown in Table 18.

Both SELEX "A" and "B" had a ligand that was more frequent in occurrence than the others. In SELEX "A" the most frequent ligand was represented by SW8.4 (SEQ ID NO: 231). This clone or closely related ligands were present in 13 of 27 (48%) gag-binding sequences obtained. In SELEX "B", the most frequent ligand was ML8.7 (SEQ ID NO: 255). This clone or closely related ligands were represented in 9 of 21 (42%) gag-binding sequences obtained. Thus in each SELEX experiment about 40-50% of the clones were a single dominant ligand. The most frequent clones from each of the two SELEX experiments were not highly similar to each other (17/50 evolved bases when optimally aligned). This was expected since different templates and conditions were used in the two SELEXs.

B. Affinity of ribonucleic acid ligands that bind to the HIV-1 gag protein.

The nucleic acid ligands (SEQ ID NOS:268-276) that bound specifically and to a significant extent to GST-gag and not to nitrocellulose filters were analyzed further over a wider range of GST-gag concentrations to obtain an apparent binding affinity (K_d). These results are shown in Table 17. Most of the ligands bound to gag with a K_d near 1-10 nM. Ψ -456 typically bound to GST-gag with a K_d near 1 nM. Therefore, the gag nucleic acid ligands can bind to gag at least as well as the native HIV-1 Ψ element.

Some of the affinity of Ψ -456 for GST-gag could be due to a length affect. We attempted to use shorter forms of Ψ -456 that were closer in length to that of the GST-gag ligands (97-98 bases). Forms of Ψ -456 were generated that were 70, 93, or 136 bases long (see Example 13), but these did not bind as well as Ψ -456 to GST-gag (K_d = 500, 500, and 10 nM respectively) or to the same extent. Therefore, Ψ -456 was used in the various binding and competition binding experiments reported here.

C. Binding of nucleic acid ligands to different sites on the HIV-1 gag polyprotein.

Regions of GST-gag to which the RNA ligands bind were determined. Twenty three GST-gag ligands were screened for binding to GST, HIV-1 capsid, HIV-1 nucleocapsid (GST-p7 or GST-p15), and HIV-1 matrix proteins. The binding specificity of the nucleic acid ligands that bind to the gag protein fell mainly into two classes, those that bind matrix and those that bind to nucleocapsid (GST-p7) (Table 18).

Binding to nucleocapsid was expected since it is a known RNA binding protein. Binding to matrix was considered to be possible since the avian leukosis retrovirus

matrix protein was reported to bind RNA (Leis *et al.* (1980) J.Virol. 35:722-731) and also because the HIV-1 matrix protein is basic (pI=9.9). In the NMR structure of matrix (Massiah *et al.* (1994) J.Mol.Biol. 244:198-223); Matthews *et al.* (1994) Nature 370:666-668) there is a basic patch of amino acids near the amino terminus. These amino acids are involved in the nuclear import of the integration machinery. It is possible that the matrix nucleic acid ligands bind to this region.

One nucleic acid ligand, ML8.11 (SEQ ID NO. 256), bound to GST. In addition one ligand, ML6.17 (SEQ ID NO. 276), bound to GST-gag with high affinity, but did not bind to the p17 matrix, p24 capsid, GST-p7 (nucleocapsid), GST-p15 (nucleocapsid-p6), or GST proteins. It is possible ML6.17 binds to more than one protein component within GST-gag. This would explain its inability to bind to the separate protein components of GST-gag.

D. Frequency of matrix and nucleocapsid ligands evolved in two separate SELEX experiments.

Nucleocapsid ligands were evolved more frequently in SELEX "A" and matrix ligands were evolved more frequently in SELEX "B" (see Table 18). In SELEX "A" nucleocapsid ligands represented 74% (20 of 27) and matrix ligands represented 26% (7 of 27) of the total HIV-1 gag ligands. In SELEX "B" matrix ligands represented 56% (10 of 18) and nucleocapsid ligands represented 33% (6 of 18) of the total. This can be explained because heparin was used as a competitor of nonspecific or lower affinity protein/RNA interactions in SELEX "B". As is the case for RNA, heparin also has a higher affinity for the HIV-1 nucleocapsid protein than for the HIV-1 matrix protein. Having heparin present in SELEX "B" may have blocked an RNA binding site on the HIV-1 nucleocapsid protein to allow more frequent evolution of RNAs that bind to an RNA binding site on the HIV-1 matrix protein.

Heparin and possibly other polyanionic molecules (e.g., tRNA) can be used to manipulate the proportion of matrix and nucleocapsid ligands that are evolved using the SELEX method. In this manner one could increase the efficiency at which either matrix or nucleocapsid ligands are isolated. Furthermore, if one desired to use the Ψ element as a competitor in a SELEX experiment, for example, to attempt to improve the affinity or specificity of nucleocapsid ligands, it would be necessary to use the nucleocapsid protein

rather than gag. If gag were used in such an experiment, Ψ would bind to the nucleocapsid nucleic acid binding site and one would risk obtaining only matrix ligands.

D. Competition between HIV-1 matrix and HIV-1 nucleocapsid nucleic acid ligands for binding to the HIV-1 gag polyprotein.

5 Since ligands were evolved that bind to two separate sites on the HIV-1 gag protein (matrix and nucleocapsid), it was investigated whether ligands that bound to the separate sites would compete with each other for binding. Competition binding reactions were done between matrix ligands, nucleocapsid ligands, and Ψ -456. Homologous ligands interfered with each other for binding to gag. That is, a matrix ligand (SW8.27, SEQ ID NO:236) interfered with the binding of another matrix ligand (SW10.12, SEQ ID NO:242) and a nucleocapsid ligand (SW8.4, SEQ ID NO:231) interfered with the binding of another nucleocapsid ligand (SW8.24, SEQ ID NO:234). This indicates there may be only one binding site for RNA on matrix or nucleocapsid, but that there are at least two RNA binding sites on the gag protein. All ligands competed off the binding of the starting pool of RNA (SEQ ID NO:225) at lower concentrations.

15 On the other hand, heterologous ligands did not interfere with each other for binding to gag. A matrix ligand (SW 8.27, SEQ ID NO:236) did not interfere with the binding of a nucleocapsid ligand (SW 8.4, SEQ ID NO:231) or Ψ -456. Conversely a nucleocapsid ligand (SW 8.4, SEQ ID NO:231) did not interfere with the binding of a matrix ligand (SW 8.27, SEQ ID NO:236). Therefore at least some matrix and nucleocapsid ligands may be able to bind to gag simultaneously. This could provide an opportunity for simultaneous, combination delivery of matrix and nucleocapsid ligands to the same cell in order to effect a treatment for HIV-1 infection. That matrix ligands and nucleocapsid ligands bind to different sites on gag might be expected since matrix and nucleocapsid are at opposite ends of an elongated protein (Nermut *et al.* (1994) Virology 198:288-296).

25 It is notable that a HIV-1 nucleocapsid nucleic acid ligand does interfere with the binding of Ψ -456 to the gag polyprotein. This might be expected since Ψ is known to bind specifically to the HIV-1 nucleocapsid protein. Since HIV-1 nucleocapsid nucleic acid ligands interfere with the binding of Ψ , nucleocapsid-specific nucleic acid ligands derived by the SELEX method can function as "decoys" (i.e., compete with Ψ for

30

binding to the nucleocapsid protein) and therefore can be useful in the treatment of HIV-1 infection.

E. Binding of nucleic acid ligands to nucleocapsid proteins from diverse HIV-1 strains.

The ability of nucleic acid ligands to bind to nucleocapsid protein from different HIV-1 strains was investigated by binding nucleic acid ligand ML6.6 (SEQ ID NO.:271) to a HIV-1 nucleocapsid protein from either a macrophage-tropic MN or a T-cell tropic LAI strain of HIV-1. These two proteins differ by 3 amino acids out of a total of 55. The ligand bound best to the LAI form of nucleocapsid and had about a 10 fold lower affinity for the MN form of the HIV-1 nucleocapsid protein. This indicates there may be some preference for a nucleic acid ligand to bind to the nucleocapsid protein it was evolved to bind to, but that they can also bind to other nucleocapsid proteins. Because nucleocapsid is a small, highly conserved protein, it is thought to be less likely to mutate so as to become resistant to inhibitors that bind to it (Rice *et al.* (1995) Science 270:1194-1197). Thus, drugs that bind to the HIV-1 nucleocapsid protein may be particularly useful for treating HIV infection.

F. Minimal sequence required for nucleic acid ligands to bind to the HIV-1 gag polyprotein.

In order to learn more about what sequences might be in common among matrix or nucleocapsid ligands that might enable them to bind to gag and what the structural basis for binding to gag might be, the minimal sequences were determined that are required for some of the ligands to bind to the HIV-1 gag protein with high affinity. This information was obtained through boundary determination and truncation studies. The results of these experiments are shown in Tables 19 and 20.

Boundary experiments were performed on nine nucleocapsid ligands and three matrix ligands. The lengths of the predicted minimal ligands range from 14 to 59 bases. Seven of the ligands required part of the fixed region sequence to bind to gag, while five required only evolved sequences to bind to gag.

G. Structural basis for recognition of HIV-1 nucleocapsid protein by nucleic acid ligands.

The minimal nucleocapsid binding sequences (Table 19) all form relatively stable ($\Delta G = -2$ to -20 Kcal/mol) stem-loop structures. In addition, the boundaries of all nine

nucleocapsid ligands examined end at a palindromic sequence that ranges in size from 8-16 bases. Usually such palindromes are at the 3' boundary. The majority of the palindromes were 8-11 bases in length. Some ligands contain more than one palindrome. In such cases, it is the palindrome that is near 8-11 bases long that is near the boundary.

5 Often a UGU-purine sequence is also found near the palindrome, but in some cases this sequence is not absolutely required for high affinity binding to nucleocapsid. Many of the palindromes contain smaller 4-base palindromes at their ends. This suggests the nucleic acid ligands that bind to the HIV-1 nucleocapsid protein may interact through palindromes to form dimers. Of course, the stem-loop structures could also anneal to

10 form dimers. The exact role of the palindrome in binding of ligands to nucleocapsid is not known, but their presence in all nucleocapsid ligands indicates they are important. The presence of palindromes fits well with the documented complementary strand annealing properties of nucleocapsid (You and McHenry (1994) *J.Biol.Chem.* 269:31491-31495; Lapadat-Tapolsky *et al.* (1995) *Nuc.Acids Res.* 23:2434-2441).

15 However, it is not known if the nucleic acid ligands reported here form dimers in solution in the absence of nucleocapsid or whether nucleocapsid enhances the rate of their formation.

The binding properties of several truncates of SW8.4 (SEQ ID NO.:231) support the theory that palindromes are involved in the binding of the nucleocapsid ligands to

20 gag and nucleocapsid. Ligands that do not contain palindromes (e.g., matrix ligands) do not bind to nucleocapsid. Also ligands with mutations in the palindrome do not bind to nucleocapsid (see below).

The occluded site size of nucleocapsid can vary from about 7-15 bases, depending on the buffer conditions and the protein/nucleotide ratio (Khan and Giedroc (1994) *J.Biol.Chem.* 269:22538-22546; You and McHenry (1994) *J.Biol.Chem.* 269:31491-31495). This size is strikingly similar to the size of the palindromes observed

25 in the sequences evolved here. Six of the nine nucleocapsid ligands have palindromes that are 8-11 bases long. This lends further support that the palindromes observed in the nucleocapsid ligands are of significance with respect to binding to the HIV-1 gag

30 polyprotein. Thus the sequences of nucleic acid ligands that bind to gag appear to be

most representative of the initial interactions between nucleocapsid and the Ψ region *in vivo* (annealing of complementary strands between the loop region of kissing loops).

Alternative nucleocapsid binding motifs that were considered include guanine quartets (Sundquist and Heaphy (1993) *Proc.Natl.Acad.Sci.USA* 90:3393-3397) and kissing loops (Paillart *et al.* (1994) *J.Biol.Chem.* 269:27486-27493); Skripkin *et al.* (1994) *Proc.Natl.Acad.Sci.USA* 91:4945-4949); Laughrea and Jetté (1994) *Biochemistry* 33:13464-13474; Muriaux *et al.* (1995) *J. Biol. Chem.* 270:8209-8216; Laughrea and Jetté (1996) *Biochemistry* 35:1589-1598), which have been proposed to be involved in the binding of Ψ to gag. There has been some controversy about what the important sequence elements are for recognition of Ψ by gag and for RNA packaging.

Different elements may be required for distinct dimer formation, gag binding, and RNA packaging steps. The involvement of guanine quartets was ruled out as a mechanism for the binding of HIV-1 nucleocapsid nucleic acid ligands because there were no consistent sequence patterns found in all HIV-1 nucleocapsid nucleic acid ligands that would suggest guanine quartets could form. In addition, the binding of one nucleocapsid ligand (SW8.4; SEQ ID NO. 231) to GST-gag was not sensitive to salt as would be expected if guanine quartets were involved. SW8.4 bound to gag as well in lithium or sodium as in the potassium used in the SELEX combinatorial chemistry procedure. Potassium has been reported to dramatically stabilize guanine quartets.

It was possible that the binding of ligand SW8.4 involved a kissing loop since a sequence within SW8.4 (positions 29-55) can form a kissing loop-like structure that is structurally similar to the proposed kissing loop in Ψ . However, the involvement of kissing loops in the binding of SW8.4 to the HIV-1 nucleocapsid protein was ruled out because the boundary of SW8.4 does not include the entire kissing loop and the truncated SW8.4t30-53 ligand, which does not include all the kissing loop, still binds to nucleocapsid.

H. Structural basis for recognition of HIV-1 matrix protein by nucleic acid ligands.

A common sequence motif could be discerned among the three minimal HIV-1 matrix nucleic acid ligands examined. The consensus sequence motif is:

PuXPyAGX₆PuGXPuXCAXPuXPuPuXGXUGXA (SEQ ID NO:289)

The entire sequence of each minimal matrix ligand is predicted to form a stable stem/loop structure. The CAXPu motif is found in the predicted loop region of each. With the exception of matrix ligand ML8.7 (SEQ ID NO. 255), other matrix ligands (including SW8.27 (SEQ ID NO. 236) which was not amenable to boundary
5 determination) do not contain palindromes. The palindrome within the boundary of ML8.7 does not fit the size and position observed for those found in nucleocapsid ligands. Therefore, the sequence and structural motifs required by matrix-specific nucleic acid ligands for binding to gag are different from those required by nucleocapsid specific ligands.

10 1. *Mutated HIV-1 gag nucleic acid ligands.*

Mutations in two ligands were constructed in order to study the role of putative sequence elements in their ability to bind to the HIV-1 gag protein.

As discussed above, the matrix ligands are predicted to form a stem/loop structure with a CAXPu motif near the loop. Although the role of sequences within the matrix
15 motif in GAG binding have not been studied extensively, deletion of a GC dinucleotide overlapping the CAXPu motif (GCAAG) in ligand ML6.8 (SEQ ID NO. 272) eliminated binding to matrix (see Table 21). This supports the notion that conserved nucleotides in the consensus matrix ligand sequence are important and that sequences in the loop may be particularly important.

20 Two versions of ligand SW8.4 (See Table 21) were constructed and tested for binding to GST-p7 (see Table 21). Ligand SW8.4G47C is a full length version of SW8.4 that has a single point mutation at position 47. This mutation is in the palindrome proposed to be involved in recognition of the nucleocapsid protein. This mutation eliminated the ability of SW8.4 to bind to nucleocapsid. Ligand SW8.4 GUCCAU
25 (45-50) GCGCGC was constructed to determine if replacement of the proposed loop (GUCCAU) in the proposed kissing loop of SW8.4 by the sequence (GCGCGC) of the proposed kissing loop of Ψ would still allow SW8.4 to bind to GST-gag. This mutation is a triple mutant relative to the wild type sequence. In addition, since the loop is also the same as the palindrome proposed to bind nucleocapsid, this mutant also introduces a
30 triple mutation into the palindrome. This triple mutant also eliminated the ability of SW8.4 to bind to nucleocapsid.

J. *Truncated nucleic acid ligands that bind to the HIV-1 gag protein.*

Nine truncated nucleic acid ligands were generated based on the boundary data shown in Table 19 in order to validate the results of the individual 5' and 3' boundary experiments. These were tested for binding to the appropriate HIV-1 protein. The results are shown in Table 20. Six truncated nucleic acid ligands were identified. Five of these bind to the HIV-1 nucleocapsid protein and one binds to the HIV-1 matrix protein.

All five truncated nucleocapsid ligands which correspond to the predicted minimal boundaries bind to GST-p15 nucleocapsid. The smallest truncate tested that bound to nucleocapsid was 29 bases long, but in theory a 14 base long truncate of ligand SW8.25 (SEQ ID NO. 235) may be able to bind to nucleocapsid.

Two truncates of ligand SW8.4 were used to distinguish between the kissing loop model and a palindrome model for ligand binding. Ligand SW8.4_{NC}t41-55 (SEQ ID NO. 283) contains the top stem/loop of the predicted kissing loop, but does not bind to nucleocapsid. Ligand SW8.4_{NC}t30-53 (SEQ ID NO. 284) is missing the entire 3' sequence of the stem of the predicted kissing loop and thus would not be able to form a kissing loop. Nevertheless, it does bind to nucleocapsid, possibly because it contains the entire palindromic region and includes the predicted minimal ligand based on boundary experiments.

One truncated matrix ligand which corresponds to the predicted minimal boundaries binds to the HIV-1 matrix protein. This truncate tested that bound to matrix was 50 bases long.

EXAMPLE 15 - MODIFIED 2'-NH₂ PYRIMIDINE RNA LIGANDS TO HIV-1 GAG.

In order to generate ligands with improved stability *in vivo*, an experiment is carried out with randomized RNA containing NH₂ functionalities at the 2'-position of each pyrimidine. A library of 7.9×10^{14} molecules is generated that contains 50 nucleotides of contiguous random sequence flanked by defined sequences. Defined nucleotide sequences in the flanking regions of the template can serve as primer annealing sites for PCR and with one of the primers providing the T7 promoter sequence (a restriction site can be added for cloning). The random and fixed nucleotides of the

initial candidate mixture are comprised of 2'-NH₂ pyrimidine bases. The round of selection and amplification are carried out as described supra in Examples 13-14 using art-known techniques.

TABLE 1
SELEX CONDITIONS

Random RNA

SEQ ID NO:	Name	Sequence
1	30N1 RNA	5' gggagcucagaaacgcucuaa-30N-uucgacaugagggcccggaucggc 3'
2	N1 5' Primer	5' ccgaagcttaatacgaactctataggagctcagaataaacgtcaa 3'
3	N1 3' Primer	5' gccggatccggggcctcatgtcgaa 3'
4	50N7 RNA*	5' gggaggacgaugcgg-50N-cagacgacucgcccga 3'
5	N7 5' Primer	5' taatacgactcactataggaggacgatgcgg 3'
6	N7 3' Primer	5' tcgggcgagtcgctcg 3'
7	50N9 RNA*	5' gggaaaagcgaaucuaacacaaga-50N-gcuccgccagagaccacaggagaa 3'
8	N9 5' Primer	5' taatacgactcactatagggaaaagcgaatcacacaaga 3'
9	N9 3' Primer	5' ttctcggttggtctctggcggagc 3'

*All C and U have 2'NH₂ substituted for 2'OH for ribose

2'OH SELEX CONDITIONS

30N1 SELEX SELEX	Round	[sPLA ₂]	[RNA]	[tRNA]
Control	1-14	240 nM	2.4 μM	-
Standard	1-5 6-12	240 nM 12 nM	2.4 μM 120 nM	- -
Competition	1-3 4-14	240 nM 240 nM	2.4 μM 2.4 μM	- 0.5 mM

TABLE 1 (PAGE 2)

2'-NH₂ SELEX CONDITIONS
50N9 & 50N7 SELEX

IMMOBILIZED sPLA₂ PARTITIONING

Round	sPLA ₂ (pmol)	RNA (pmol)	Gel Vol	Total Vol	TBSC Eluted RNA	Ab Eluted RNA
1	0.2 - 2	468	1 ul	0.5 ml	2.6†	1.9†
2	0.2 - 2	100	1 ul	1.0 ml	3.0†	2.6†
3	0.4 - 4	345	2 ul	1.0 ml	0.1†	0.03†
4	0.4 - 4	540	2 ul	1.0 ml	0.05†	0.03†
5	2 - 20	730	10 ul	1.0 ml	0.07†	0.03†
8	2 - 20	90	10 ul	1.0 ml	1.0†	0.2†
9	2 - 20	102	10 ul	0.5 ml	2.4†	0.3†

NITROCELLULOSE PARTITIONING

Round [sPLA ₂]	[RNA]	Vol	Net RNA bound
6	25 nM	373 nM	1.5 ml
7	10 nM	100 nM	1.5 ml
10	5 nM	50 nM	1.5 ml
11	5 nM	50 nM	1.5 ml

TABLE 2*

Family I
• Ligands

10	71	gggagcucagaaauaacgcucaa	-----UCUCAU-GCUCUGCGCACGGCGUAACCUAUuucgacaugaggcccggauccggc
11	2 (6)	gggagcucagaaauaacgcucaa	-----UCUCAUUGCUCUGCGCACGGCGUAACCUAUuucgacaugaggcccggauccggc
12	3	gggagcucagaaauaacgcucaa	-----AGCUCAUUCGUC--UCGCAAGGCGUAUCCUAUuucgacaugaggcccggauccggc
13	77	gggagcucagaaauaacgcucaa	-----UUGCGGGCGUAUCCUAU-ucgacaugaggcccggauccggc
14	93 (3)	gggagcucagaaauaacgcucaa	-----CAGCCA-AU-GUGUCCCGUACGGCGUAUCCUAU-ucgacaugaggcccggauccggc
15	95	gggagcucagaaauaacgcucaa	-----CGCUGGAU-ACCA-UCGCACGGCGUAUCCUGCuucgacaugaggcccggauccggc
16	85	gggagcucagaaauaacgcucaa	-----AUGCAUCAU-GUA--CGCAAGACGUAUUCUAU-ucgacaugaggcccggauccggc
17	18	gggagcucagaaauaacgcucaa	-----AUCGCAUCAU-GUA--CGCAAGACGUAUUCUAU-ucgacaugaggcccggauccggc
18	54	gggagcucagaaauaacgcucaa	-----AUGCAUCAU-GUA--CGCAAGACGUAUUCUAU-ucgacaugaggcccggauccggc
19	14	gggagcucagaaauaacgcucaa	-----AUGCAUCAU-GUA--CGCAAGACGUAUUCUAUuucgacaugaggcccggauccggc
97		Consensus Sequence	YMUCAU-GUH--YGYAMGRCGUAUYCUAU
		Boundary Species	
101	2		CUCAUUGCUCUGCGCACGGCGUAACCUAUuucgacauga
102	Truncates and Derivatives		GG-----UCUCAUUGCUCUGCGCACGGCGUAACCUAUuucgacaugag
	2.3		

Family II
• Ligands

20	72 (6)	gggagcucagaaauaacgcucaa	-----CUUACAG-----CCCG-----GCUGAGACAC-----uucgacaugaggcc cggauccggc
21	72F	gggagcucagaaauaacgcucaa	-----CUUACAG-----CCCG-----GCUGAGACAC-----uucgacaugaggcc cggauccggc
22	96 (2)	gggagcucagaaauaacgcucaa	-----CUUACAG-----UUCG-----GCUGAGACAC-----uucgacaugaggcc cggauccggc
23	13t	gggagcucagaaauaacgcucaa	-----GUAU-----GCCGAGACAC-----uucgacaugaggcc cggauccggc
24	94	gggagcucagaaauaacgcucaa	-----CUUACGG-----UUCG-----CCCAGAUAA-----uucgacaugaggcc cggauccggc
25	15	gggagcucagaaauaacgcucaa	-----CUUACAG-----CCCG-----GCUGAGACAU-----uucgacaugaggcc cggauccggc
26	79	gggagcucagaaauaacgcucaa	-----CUUACAG-----CUCG-----GCUGAGACAC-----uucgacaugaggcc cggauccggc
27	88	gggagcucagaaauaacgcucaa	-----CUUACAG-----UCCG-----GCUGAGACAC-----uu cgacaugaggcccggauccggc
28	81	gggagcucagaaauaacgcucaa	-----CUUACAG-----CCCG-----GCUGAGACGC-----uucg acaugaggcccggauccggc
29	68t	gggagcucagaaauaacgcucaa	-----CUUACGG-----CUAAU-----GCCGAGACGC-----uucg acaugaggcccggauccggc
30	75	gggagcucagaaauaacgcucaa	-----CUUACAG-----CCCG-----GCUGGACAC-----uucg acaugaggcccggauccggc
31	73t	gggagcucagaaauaacgcucaa	-----CUUACAG-----UUCG-----GCUGAGAGAGACGCAUAUcugacaugagg cccggauccggc

TABLE 2 (PAGE 2)

32	73tF	gggagcucagaaauaaacgcucuaa	CUUACAG	-----UUCG	-----GCUGAGAGAAAGACGCAUAUcucgacauagagg	cccggauccggc
33	72t (2)	gggagcucagaaauaaacgcucuaa	CUUACAG	-----UUCG	-----GCUGAGAGAGAAAGACGCAUAUcucgacauagagg	cccggauccggc
34	71t (3)	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
35	1t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
36	11t (2)	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
37	11tF	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
38	36t (9)	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
39	4 (6)	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
40	35t (3)	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
41	89	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
42	84t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
43	79t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
98	Consensus Sequence	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
	Boundary Species	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
103	72	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
104	72t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
	Truncates and Derivatives	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
105	72.c	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
106	72t.2c	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
	Family III	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
	• Ligands	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
44	34t (2)	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
45	9t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
46	6t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
47	76t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
48	83t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
49	86t	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
50	86tF	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc
99	Consensus Sequence	gggagcucagaaauaaacgcucuaa	CUUACAG	-----GAGAUUCCAUUCGCGAGACGC	-----uucgacauagagg	cccggauccggc

TABLE 2 (PAGE 3)

Family IV
• **Ligands**
51 2t gggagcucagaauaaacgcuaaUCCGGGAGCUGAAAAA-CAUGCCGUAGCCGuucgacaugaggcccggauccggc
52 70t gggagcucagaauaaacgcuaaUCCGGGAGCUGAAAAA-CAUGCCGUAGCCAuucgacaugaggcccggauccggc

gggagcucagaauaaacgcuaaGCUUGGGAUGAUGCCAGUGUCCAGCAUCuucgacaugaggcccggauccggc
gggagcucagaauaaacgcuaaAGCUCUGGGAUGAUGCCACUGUCCAGCAUCuucgacaugaggcccggauccggc
gggagcucagaauaaacgcuaaGCUUGGGAUGAUGCCACUGUCCAGCAUCuucgacaugaggcccggauccggc

Boundary Species
107 80 gggagcucagaauaaacgcuaaGCUUGGGAUGAUGCCAGUGUCCAGCAUCuucgac
108 87.3 gggagcucagaauaaacgcuaaAGCUCUGGGAUGAUGCCACUGUCCAGCAUCuucgac

Truncates and Derivatives
109 87.3 gggagcucagaauaaacgcuaaGCUUGGGAUGAUGCCACUGUCCAGCAUCuucgacaug
110 87.4 gggagcucagaauaaacgcuaaGCUUGGGAUGAUGCCACUGUCCAGCAUCuucgacaug
111 87.5 gggagcu----auaaacgc---agCUCUGGGAUGAUGCCACUGUCCAGCAUCuucgacaug
112 87.7 gggagcucagaauaaacgcuaaGCUUGGGAUGAUGCCACUGUCCAGCAUCuucgacaugUGUAGCUAAACAGCUUUAAGGA

Orphan Sequences
• **Ligands**
56 1 gggagcucagaauaaacgcuaaGCGGAACCGAUAUGGAGGGAUUGCGuucgacaugaggcccggauccggc
57 84 gggagcucagaauaaacgcuaaGACACACGUCGGAACGAACACCGCCACGCAuucgacaugaggcccggauccggc
58 90 gggagcucagaauaaacgcuaaCCAACGACACUCACGCAUUGCCACGAAAGGuucgacaugaggcccggauccggc
59 60 gggagcucagaauaaacgcuaaACAACGCGCCACACGCGGAGAUCCGAGAAAGGuucgacaugaggcccggauccggc

Family VI
• **Ligands**
60 NN11 (5) gggaaaagcgaaaucauacacaagaCCGGCCGGGGAAA---CCCGAGGUCCGAGGUACGCAUGGCGCCUCACCGAGUCgucuccgagaa agaccaaccgagaa
61 NN27 gggaaaagcgaaaucauacacaagaCCGGCCGGGGAAA--CCCGAGGUCCGAUGUAACGCAUGGCGCCUCACCGAGUCgucuccgagag accaaccgagaa
62 NN16 gggaaaagcgaaaucauacacaagaCCGGCCGGGGAAA---CCCGAGAUCCGAGGUACGCAUGGCGCCUCACCGAGUCgucuccgagag accaaccgagaa
63 NN10 gggaaaagcgaaaucauacacaagaCCGGCCGGCGCCAUUA-GCCGAGAUCCGAGGUUGUACGAUGACAAUCACAGUgucuccgagagacca accgagaa
64 NN19 gggaaaagcgaaaucauacacaagaCCGGCCGGGGCCAUUA-GCCGAGAUCCGAGGUUGUUAACGAUAACUCGGUGucuccgagagaccaa cggagaa
65 NN2 (4) gggaaaagcgaaaucauacacaagaGACACCGCCAGCCCAAGGC-UCUAGAUUCCAGGUUUCAGAAACCCCAUCGGGUUGgucuccgagagaa ccaaccgagaa

TABLE 2 (PAGE 4)

66 NN11d gggaaaagcgaaucacacaaagaCCGGCCCGUAUAGC'CGAGAUCCGAGACUUCGUAACGAGGUGCCACGgcuccgccagagacc aaccgagaa
 67 NN29 gggaaaagcgaaucacacaaagaCCGGCCCGUGUGUAGCCGGAGAUCCGAGACUUCGUAACGAGGUGCCACGgcuccgccagagacc aaccgagaa
 68 NN41 gggaaaagcgaaucacacaaagaCCGGCCCGUGUGAGCCGGAGAUCCGAGACUUCGUAACGAGGUGCCACGgcuccgccagagacc aaccgagaa
 69 NN5 (2) gggaaaagcgaaucacacaaagaCCGGCCCGCCCAUUAAGGAGAUCCGAGGAAUUGAAUUGUUGAGUGAGcuccgccagagacc aaccgagaa
 100 Consensus Sequence ragaCCGGCCNGSNNNNNNNSCNGAGAUCCGAGG

 Boundary Species
 113 NN11 aagaCCGGCCGGGAAA--CCCGAGGUCCGAGGUAAACGCA
 114 NN19 aagaCCGGCCGGCCCAUA-GCCGAGAUCCGAGGUGUUG
 115 NN2 gaGACCGGCCAGCCAAAGGC-GCUGAGAUCCGAGGUTUCA
 116 NN41 aagaCCGGCCCGUGUGCAGCCCGAGAUCCGAGACTUUCUG

 Truncates and Derivatives
 117 NN19.4 gggaaaag-----aagaCCGGCCGGCCCAUA-GCCGAGAUCCGAGGUGUUGA
 118 NN19.11 gggaaaag-----aagaCCGGCCGGCCCAUA-GCCGAGAUCCGAGGUGUUGAACGAU-----agac caaccgagaa
 119 NN19.13 gggaaaag-----aagaCCGGCCGGCCCAUA-GCCGAGAUCCGAGGUGUUG-----agac caaccgagaa
 120 NN19.14 gggaaaag-----aagaCCGGCCGGCCCAUA-GCCGAGAUCCGAGGUGUUGAACGAU-----ccgagaa
 121 NN19.15 gggaaaag-----aagaCCGGCCGGCCCAUA-GCCGAGAUCCGAGGUGUUG-----ccgagaa
 122 NS2 (9) gggaggacgaugcggUAUUAACGGA----UACUGAUCCGAGGUUAUAGUCAUAUAUACCGCGUGCGCgagacgacgccccga

 Other Sequences
 *
 70 NN3 gggaaaagcgaaucacacaaagaGUGUGCGUGCAUGCGUGGUAAGGGGGUGGGAAGAAAACCGGCCgcuccgccagagacca accgagaa
 71 NN4 (9) gggaaaagcgaaucacacaaagaGUGUGCGUGCAUGCGUGGUAAGGGUGGUGUAGGGAAGACCGUGCCgcuccgccagagacca accgagaa
 72 NN1 gggaaaagcgaaucacacaaagaCGGCGAGCAUCCGGCGAGUGGAGGGGACUGAUGGAGGGCGAGACCUGUGgcuccgccagagacca accgagaa
 73 NN6 gggaaaagcgaaucacacaaagaCGGCGAGCAGCGCGGAGUGGAGGGAGGACUGAUGGAGGGCGAGCCGCAAGcuccgccagagacca accgagaa
 74 NN7 gggaaaagcgaaucacacaaagaCGGCGAGCAAGCGCGAGUGGAGGGAGGACUGAUGGAGGGCGAGCCGCAAGcuccgccagagacca accgagaa
 75 NN22 gggaaaagcgaaucacacaaagaCGGCGAGCAUCCGGCGAGUGGAGGGAGGAGACUGAUGGAGGGCGAGACCUGUGgcuccgccagagacca accgagaa
 76 NN45 gggaaaagcgaaucacacaaagaCGGCGAGCAGCGCGCGAGUGGAGGGAGGAGACUGAUGGAGGGCGAGACCUGUGgcuccgccagagacca accgagaa
 77 NN40 (2) gggaaaagcgaaucacacaaagaCCCUAGUCUUGAGUCGACGAGGAGGAGGGCCGAGGAGGAAGUCCGUCAGGAGcuccgccagagacca accgagaa
 78 NN18 gggaaaagcgaaucacacaaagaGGAUGGCGGCAAGGCGCGAAGGGAGGAUCCGAGGAGGAUCCGUCAGGAGcuccgccagagacca caaccgagaa
 79 NN30 gggaaaagcgaaucacacaaagaCCACGCGAGUGUCGACAGUGUGGGUGGAAGUGACGGAGAGGAUUGGAGAC'Gcuccgccagagacca accgagaa
 80 NN39 gggaaaagcgaaucacacaaagaCUGGUUGUGCGGACCCAGUCAGUGGGAGGAC'GUGAGGUGGCGAGCUUGGgcuccgccagagaccaac cgagaa

TABLE 2 (PAGE 5)

81	NS20 (17)	gggaggacgaugcggaggguagcggagggaagaaacgaucguguguaacccgaacggaucguggycagacgacucgccc a
82	NS39 (3)	gggaggacgaugcggguagggaugaaucggguguaucgggaugauagugucccagacgacucgccc a
83	NS38 (2)	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
84	NS4	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
85	NS12 (2)	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
86	NS27	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
87	NS11 (2)	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
88	NS1	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
89	NS49	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
90	NS48	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
91	NS13	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
92	NS10	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
93	NS14	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
94	NS6	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
95	NS18	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a
96	NS7	gggaggacgaugcggguagggaugaaucggggaagagagacacacacggaagagagacacacggaagagagacgccc a

*Fixed sequences are represented by lower case lettering; evolved sequence by upper case. "-" indicates spacing for alignment. Ligands recovered from the 2'OH Competition SELEX are denoted by "t"; F indicates 2'OH ligands which contain 2'F CTP and 2'F UTP in place of CTP and UTP, respectively. Sequences that were isolated more than once, are indicated by the parenthetical number, (n), following the ligand isolate number.

• Sequence ID Number

TABLE 3 (Page 1)

A: 2'OH Ligands

Ligand	SEO ID NO	Kd1	Kd2	Mole fraction
--------	-----------	-----	-----	---------------

Family I

71	10	1.3 nM	82 nM	0.60
2	11	1.7 nM	180 nM	0.45
2.3	102	2.6 nM	740 nM	0.55
3	12	1.9 nM	33 nM	0.55
85	16	3.8 nM	92 nM	0.60
93	14	17.2 nM	82 nM	0.50
95	15	4.5 nM	140 nM	0.65
18	17	5.0 nM	240 nM	0.50
54	18	16.6 nM	350 nM	0.70

Family II

72	103	0.5 nM	270 nM	0.13
72F	21	5.6 nM		
72c	104	4.6 nM	170 nM	0.15
96	22	0.9 nM	130 nM	0.40
79	26	1.7 nM	52 nM	0.35
73t	31	2.4 nM	530 nM	0.40
73tF	32	14.0 nM		
75t	30	0.9 nM	420 nM	0.40
72t.2c	106	5.0 nM	360 nM	0.40
11t	36	0.6 nM	110 nM	0.40
11tF	37	7.0 nM		
4	39	0.7 nM	300 nM	0.55
89	41	2.5 nM	62 nM	0.50
35t	40	2.4 nM	170 nM	0.65

Family III

9t	45	39.4 nM	310 nM	0.25
6t	46	28.0 nM	1830 nM	0.50
83t	48	10.5 nM	170 nM	0.30
86t	49	2.8 nM	450 nM	0.35
86tF	50	12.0 nM		

Table 3 (Page 2)

<u>Ligand</u> <u>Family V</u>	<u>SEO ID NO</u>	<u>Kd1</u>	<u>Kd2</u>	<u>Mole fraction</u>
80	53	1.6 nM	300 nM	0.40
87	54	0.8 nM	450 nM	0.50
87F	55	1.2 nM	840 nM	0.50
87.3	109	0.8 nM	720 nM	0.40
87.4	110		4350 nM	
87.5	111	3.1 nM	1320 nM	0.50
87.7	112	0.8 nM	250 nM	0.30

A: 2'OH LigandsUnrelated Sequence

1	56	1.4 nM	43 nM	0.25
60	59	0.3 nM	200 nM	0.20

Random RNA

30N1	1		360 nM	
30N1F	1		35 nM	

B: 2'NH₂ LigandsFamily VI

NN11	60	2.8 nM		
NN27	61	0.4 nM	150 nM	0.2
NN16	62	0.9 nM		
NN10	63	0.4 nM		
NN19	64	0.4 nM		
NN2	65	1.2 nM		
NN11d	66			
NN29	67	2.5 nM		
NN41	68	1.2 nM		
NN5	69	4.6 nM		
NN19.4	117	7.3 nM		
NN19.11	118	0.5 nM		
NN19.13	119	2.2 nM		
NN19.14	120	1.0 nM		
NN19.15	121	1.7 nM		
NS2	122	22.0 nM		

Table 3 (Page 3)

<u>Ligand</u>	<u>SEQ ID NO</u>	<u>Kd1</u>	<u>Kd2</u>	<u>Mole fraction</u>
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Other Sequences

NN1	72	21 nM		
NN4	71	27 nM		
NN18	78	71 nM		
NN22	75	36 nM		
NN30	79	23 nM		
NN39	80	52 nM		
NN40	77	31 nM		

NS1	88	42 nM		
NS4	84	24 nM		
NS6	94	28 nM		

A: 2'NH₂ Ligands

NS7	96	18 nM		
NS10	92	22 nM		
NS11	87	16 nM		
NS12	85	40 nM		
NS13	91	15 nM		
NS14	93	12 nM		
NS18	95	52 nM		
NS39	82	16 nM		
NS48	90	42 nM		
NS49	89	26 nM		

Random 2'NH₂ RNA

50N7 RNA	4	71 nM		
50N9 RNA	7	48 nM		

Table 4
Selectivity of sPLA₂ RNA Ligands

K_d(nM) to specified protein*

<u>Seq. ID#</u>	<u>Ligand</u>	<u>Lysozyme</u>	<u>bFG</u>	<u>bpPLA₂</u>	<u>sPLA₂</u>
11	2	1,700	230	20,000	1.7
20	72	1,500	180	29,000	0.5
53	80	2,70	240	29,000	1.6
54	87	2,300	220	20,000	0.8
1	30N1	1,650	270	22,500	360

* Standard binding experiments were performed to the specified target protein in TBSC and K_d's calculated by curve fitting. The ligands bind no better than randomized RNA (30N1) to lysozyme (hen egg-white), human basic fibroblast growth factor basic (bFGF) and bovine pancreatic PLA₂. The sPLA₂ K_d is that of the high affinity species.

Table 5

Selectivity of sPLA₂ 2'NH₂ RNA LigandsK_d(nM) to specified protein*

<u>Seq. ID#</u>	<u>Ligand</u>	<u>Elastase</u>	<u>bFGF</u>	<u>C1q</u>	<u>IgG</u>	<u>sPLA₂</u>
64	NN19	40	210	-	>5,000	1
121	NN19.15	40	165	1,000	4,300	0.4
7	50N9	70	170	1,000	>5,00	48

* Standard binding experiments were performed to the specified target protein in TBSC and K_d's calculated by curve fitting. The ligands bind no better than randomized RNA (30N1) to human neutrophil elastase, human basic fibroblast growth factor (bFGF), and human immunoglobulin G (IgG and C1q).

TABLE 6

Starting RNA:

5'-GGGAGCUCAGAAUAAACGCUCAA[-30N-]UUCGACAUGAGGCCCGGAUCCGGC-3'
(SEQ ID NO:123)

PCR Primer 1:

Hind III

5'-CCGAAGCTTAATACGACTCACTATAGGGGAGCTCAGAATAAACGCTCAA-3'
(SEQ ID NO: 124) T7 Promoter

PCR Primer 2:

Bam HI

5'-GCCGGATCCGGGCCTCATGTCGAA-3'
(SEQ ID NO:125)

TABLE 7

SEQ ID NO.	Ligand	SEQUENCE	VEGF Protein Binding Kd	VEGF Receptor Binding Ki
126	NX-107	ACC CUG AUG GUA GAC GCC GGG GUG		1 nM
127	NX-176	ACC CUG AUG GUA GAC GCC GGG GUG	65 nM	10nM
128	NX-178 T*T*T*T*	ACC CUG AUG GUA GAC GCC GGG GUG T*T*T*T*T	0.7 nM	1 nM
129	NX-190 T*T*T*T*	ACC CTG ATG GTA GAC GTT GGG GTG T*T*T*T*T		
130	NX-191 T*T*T*T*	ACC CUG AUG GUA GAC GCC GGG GUG T*T*T*T*T	120 nM	500nM
131	NX-213 T*T*T*T*	ACC CUG AUG GUA GAC GCC GGG GUG T*T*T*T*T	0.2 nM	1 nM
132	NX-214 T*T*T*T*	ACC CUG AUG GUA GAC GCC GGG GUG T*T*T*T*T	0.2 nM	1 nM
133	NX-215 T*T*T*T*	ACC CUG A*UG GUA GAC GCC GGG GUG T*T*T*T*T	0.2 nM	1 nM
134	NX203	ACC CUG AUG GUA GAC GCC GGG GUG		
135	NX204	ACC CUG AUG GUA GAC GCC GGG GUG		
136	NX205	ACC CUG AUG GUA GAC GCC GGG GUG		
137	NX206	ACC CUG AUG GUA GAC GCC GGG GUG		

N = 2'OH N = 2'NH₂ N = 2'OMe

N* = phosphorothioate N = 2'deoxy

N = 2'OMe:2'OH::2:1

TABLE 8
EX VIVO RAT TISSUE STABILITY: BRAIN

TIME (min)	PER CENT FULL LENGTH			
	NX 107	NX 178	NX 190	NX 213
3	94.82	100.48	99.61	100.33
10	91.66	96.27	99.23	99.81
30	79.47	86.98	97.53	99.00
60	73.04	79.39	96.37	99.02

⊗

TABLE 9
EX VIVO RAT TISSUE STABILITY: KIDNEY

TIME (min)	PER CENT FULL LENGTH			
	NX 107	NX 178	NX 190	NX 213
3	90.34	96.07	99.05	100.01
10	69.97	96.55	97.13	100.13
30	46.30	92.37	94.56	99.40
60	45.00	90.14	91.83	99.09

TABLE 10

Starting Single Stranded DNAs and the Corresponding PCR Primers Used in the ssDNA SELEX Experiments Targeting VEGF

SELEX experiment A

Starting ssDNA:

5'-ATCCGCCTGATTAGCGATACT(40N)ACTTGAGCAAAATCACCTGCAGGGG-3'
(SEQ ID NO: 216)

PCR Primer 1:

5'-JJJCCCCTGCAGGTGATTTTGCTCAAGT-3' (SEQ ID NO: 217)

PCR Primer 2:

5'-ATCCGCCTGATTAGCGATACT-3' (SEQ ID NO: 218)

SELEX Experiment B

Starting ssDNA:

5'-CTACCTACGATCTGACTAGC(40N)GCTTACTCTCATGTAGTTCCT-3'
(SEQ ID NO: 219)

PCR Primer 1:

5'-AJAJAGGAACTACATGAGAGTAAGC-3' (SEQ ID NO: 220)

PCR Primer 2:

5'-CTACCTACGATCTGACTAGC-3' (SEQ ID NO: 221)

J=biotin

Table 11 - VEGF ssDNA Ligands

ssDNA bulk pool, BH SELEX: 0.44 nM			Kd,nM
SEQ ID NO:	Ligand		
Family I			
138	3	acaacggcgtggaagactagatgcagccgaacgcacatcta	1
139	5	acgtacaagtcgcgtgtggtagacaagagtgagggaag	
140	9 (3x)	agcccgctcgaagntagagcgagggcccaaaataccg	
141	10	gtaccatccacgggttacgtggacaagagggccctggtag	0.88
142	11	tcactacaagtcgcgcgtggttagacaagagtgagggaag	
143	15	accgctgtgagtcccttaggactagagggccgcctac	
144	21	taggcttgacgtcttctagactagagtgacgtcaaaccc	2
145	27	tgcaggtcgactctagaggatccccgggtaccgagctcga	
146	31	acggtttacgtggacaagagggccctggtag	
147	32 (3x)	ggtggactagaggnacagcaaacgataccttggttcgcgtcc	2
148	33	tcaagcactccgctctccagacaagagtgacggcctct	
149	35	cgtgatggacaagagggccctatccacggatatccgtc	
150	37	caagcagtgccgctctccagacaagagtgacggcctct	0.5
151	39	tgatccaccgtttatagtcggtggttagacaagagtgacagg	
152	41	aacacacagaggacagttacaggtaacatccgctcagg	
153	49	agtggcgtctatagacaagagtgacggccgagtttca	8.1
154	50	ccacaagagggcagcaagtgcacacacagcgtccgg	
155	b56 (8x)	gcagggccacgtctatttagactagagtgacgtggttc	
156	b69	acgggtccaaaggtttcccatccgtggactagagggcacgtgctta	0.3
157	b80	ccgtcgcgtgactataaccacacgcagactagagtgacgggctta	
158	b81**	ccgaatggggctgcgactgcagtcgagtcacgtcgtta	
159	b91**	acgcaagagagtcnccgaatgcagtcctcagccgctaaca	
210	Consensus	agacaagagtgacagg	
211		ggactagagggcagtg	

TABLE 11 (PAGE 2)

SEQ ID NO:	Ligand Family 2	Kd PCR
160	2	
161	14	
162	25 (2x)	
163	40	
164	46	
165	b54	2.5
166	b55	15
167	b59	91
168	b79	2
169	b81**	0.3
170	b85	0.15
171	b88 (5x)	34
172	b89 (3x)	2
173	b91**	3.9
174	b99	34
212	Consensus	
	Family 3	
175	18	
176	19	
177	b51	
178	b60	26
179	b62	2.9
180	b63	
181	b65	0.53
182	b66	1.3

TABLE 11 (PAGE 3)

SEQ ID NO:	Ligand	Kd PCR
	Family 3 (con't)	
183	b95 ccactgcatagagaactggatcgacgggtccaaagttcgggtgctta	0.9
184	b96 .ccactgcatagagatactggattcgacnnnccaaagtttcgggtgctta	1.5
185	b97 ccactgcagagagtcacacttacgangccaaaggttcgggtgctta	>1
213	Consensus cccactggatagag	
214	ccccactgcatagag	
	Family 4	
186	1 tctgcgagagacctactggaacgtttttgtgatattcaca	12
187	6 atacacccggcggtgctaccggatcggttgatttctctcc	1.0
188	13 acgccccctgagacctaccggaatntntcgtaggccta	
189	23 ggcatctaaaccagacctaccggaacgttatcgcttg	0.75
190	44 ggtgtgaaccagacctacnggaacgttatcgcttg	0.4
215	Consensus agacctaccggaacgtt	
	Orphans	
191	4 catcagtattatataacgggaaccaaacggcaaatgctgac	
192	7 tccnngggagaatagggttagtcggagaagttaatcgct	
193	16 cgggaacgtgtggttacnccgctactggattgtttcctg	
194	30 ggtagggtccggtgtgaaagaggttcgcatcaggta	
195	38 cctcagggaacatagttgagcatcgatcgatcctggag	
196	43 ttggcttgagtcctgggacgcactgttgacagtggagt	
197	45 cagcaggttagtataacgggaaccaaacggcaaatgctgac	
198	b53 gcaagggtcatctcggaatcggttaatctgacttgcaatcgctta	2.5
199	b98 gatccacgaagaagcttactctcatgtagttcca	>100

TABLE 11 (PAGE 4)

SEQ ID NO:	Ligand	Kd PCR
	Truncates	
200	10t	5
201	15t	3
202	32t	17
203	33t	0.7
204	56t	0.2
205	85t	0.3
206	88t	19
207	65t	0.32
208	66t	0.35
209	23t	>200

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gtaccatccacggtttacgtggacaagaggccctgggtac
gtagttccttttaggactagaggccgcctac
tggactagaggnacagcaaacgataccttggttcggtcc
ccggtcttccagacaagagtgcaggg
agggccacgtctatttagactagagtgcagtgggttc
ggagtgcggttgcgcgaagggtcggttagtcacctc
gcaagggtctctgcgaatggggaatacgcctagccgaaa
cgaggtccccccactggatagagttgtgaaacaacggtgcgctta
aacacttccccactggatagaggccttccgcagagccggtgctta
gggcatctaaccacacctaaccggaacgttatcgcttggtg

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TABLE 12

Starting template sequence for SELEX "A":
 5'-GGGAGACAAAGAAATAACGCTCAA-50N-TTCGACAGGAGGGCTCAACACAGGC-3' (SEQ ID NO:222)

5' PCR primer for SELEX "A":
 5'-TAATACGACTCACTATAGGGAGACAAAGAATAAACGCTCAA-3'(SEQ ID NO:223)

3' PCR primer for SELEX "A":
 5'-GCCTGTTGTGAGCCTCCTGTGCGAA-3'(SEQ ID NO:224)

Starting RNA sequence pool for SELEX "A":
 5'-GGGAGACAAAGAAUAAACGCUCAA-50N-UUCGACAGGAGGCUCACACAGGC-3' (SEQ ID NO:225)

5' primer for cloning evolved ligands from SELEX "A":
 5'-GCCGGA.TCCGCCTGTTGTGAGCCTCCTGTGCGAA-3'(SEQ ID NO:226)

3' primer for cloning evolved ligands from SELEX "A":
 5'-CCGAAGCTTAATACGACTCACTATAGGGAGACAAAGAATAAACGCTCAA-3' (SEQ ID NO:227)

primer for sequencing cloned ligands from SELEX "A" and "B":
 5' TAATACGACTCACTATA 3' (SEQ ID NO:228)

TABLE 13

Evolved *lag* ligands from SELEX experiment "A"

5'-GGGAGACAAAGAAUAAACGCUCAA-50N-UUCGACAGGAGGCCUCACAAACAGGC-3' (SEQ ID NO:225)

SEQ ID NO:	Ligand Name	Sequence of randomized region
229	SW8.1	UUGUGAUCUACCGUUAACCGGACGGUGUUUUUACACCAACGAACCUUGG
230	SW8.2	UGAACCUUGAGUUCUUAUAGAACUGAUUUCUUAAGAAAGGGGUAGAUG
231	SW8.4	CGUCUUCUACAGAGGAACCGUGGUGCAUCUGUGAAGUUGAUUCCUAGU
232	SW8.6	GGGUUGAUUACCAAAUCGUAACTUGUACCCUGCCUACACUAGGACAAACGG
233	SW8.20	CUAACCGAAGCUCUGAGAGAAUAGUUUAUCCAGUGAUGAAUCCUGAUGGG
234	SW8.24	CGAGCUUUUAGUAAGACUCAUGCCGAGAAUUCGGUCGUGAUGCUGUCGAG
235	SW8.25	GCCCCGGAAUUGCAUUGUGUGCGUGCGGGGAGUCCAAAGUCAGCAUCCUCA
236	SW8.27	GGGUUGAUUACCAAAUCGUAACTUGUACCCUGCCUACCCUAGGACAAACGA
237	SW8.30*	AUCCACUCGCGUGACCCUGACGUGAAACCGAAUAGGAUCCGACGUCACAA
238	SW8.31	GUGUCCCUUGUCACCCUGGACUGGGCCGUUUGAACUGACAUUCUAUACGA
239	SW8.37	GGCCAAACGUCGAGUUGGUUUUCCAUAGCUGAGCACAGGACGGCUUCUGC
240	SW8.40	CCCGGAUGGUGAGCCUUGUCGGAGAUUGGCACGAGGGUAAAGGGUAGGGA
241	SW10.7	CUGUUGCUAAAGUAGAAGUCAUUAUUCUGCGAUGGUUUAAGAUAACCCAGCC
242	SW10.12	UCUCGUUUCAGCACACCCGGUACAAAGAGGAUGCAAAUACGCCUUGAC
243	SW10.22	CGUCUUCUACAGGGAAACCGUGGUGCAUCUGUGAAGUUGUUGUAGAUAUCCUAGA
244	SW10.25	UGUCUUGGCGUCCACGUCUGUAGUGUGUGGGGGAAAGAGGAGGGUGCAC
245	SW10.28	UUUUUAGCCUGGUGAAGCAUGUACUGCGAAUGUGAGGGAAUGUGGAAGGG
246	SW10.34	GCUCUCCUAGCCGACUUCUUGUUGGACGAGGGUUGUACCGUGGGAGGAUG
247	SW10.39	CGAGCCUUUAGUAAGACUCAUGCCGAGAAUUCGGUCGUGAUGCUGUCGAG
248	SW10.43	UUCGUGAGGGCGGUGUGGAGGCAAGCGGUACGAGCGUACUGUCUGGGGCC

* The 5'-fixed region is missing nucleotide A in the 9th position

TABLE 14

Starting template sequence for SELEX "B":	
5'-GGGAAAAGCGAATCATACACAAGA-50N-GCTCCGCCAGAGACCAACCGAGAA-3'(SEQ ID NO:249)	
5' PCR primer for SELEX "B":	
5'-TAATACGACTCACTATAGGGAAAAGCGAATCATACACAAGA-3'(SEQ ID NO:250)	
3' PCR primer for SELEX "B":	
5'-TTCTCGGTTGGTCTCTGGCGGAGC-3'(SEQ ID NO:251)	
Starting sequence of RNA pool for SELEX "B":	
5'-GGGAAAAGCGAAUCAUACACAAGA-50N-GCUCCGCCAGAGACCAACCGAGAA-3'(SEQ ID NO:252)	
5' primer for subcloning evolved ligands from SELEX "B":	
5'-CCGCGGATCCTAATACGACTCACTATAGGGAAAAGCGAATCATACACAAGA-3' (SEQ ID NO:253)	
3' primer for subcloning evolved ligands from SELEX "B":	
5'-GGCGAAATTCCTCTCGGTTGGTCTCTGGCGGAGC-3' (SEQ ID NO:254)	

TABLE 15

Evolved gap ligands from SELEX experiment "B"

5'-GGGAAAGCGAUAUACACAAGA-50N-GCUCCGCCAGAGACCACCGAGAA-3' (SEQ ID NO:252)

SEQ ID NO:	Ligand Name	Sequence of randomized region
255	ML.8.7	UGUAUGGGUGUGUUUGGUUAGCUGUGAGGGCCAUUGGGCUGGACG
256	ML.8.11	GGUGUGUGCGCCCGAGGUGUCUGAGGAGAGAGGUGGCAGUGGAGGGU
257	ML.8.12	GGAGGGUGUGCGGACCGGAGCGGUGAGUGAGGGCUUUCAGGCGUUGGACG
258	ML.8.14	UGUGAUGGGUGUGAUUUUGGUUAGCUGUGGAGGGUUAUGUGGUCGGACG
259	ML.8.16	UGUGGGCUUCCUGAGGGGUAGGAACUCUGAAGUCAUGGUUCGUGGUAAGC
260	ML.8.17	GGAGGGAUUGUGGAAGGGUUUGUGGUGUUUCGCAAUUGCCCAUUGGACGU
261	ML.8.18	GGGGGAGAGGGCGUGGACGAUGUUUGUGGUUAUGCUGUCGGUUUUGGCUUG
262	ML.8.19	GGGAUGGAUCGGUGAGACGACGACUGGAGUGGUGAGGUGGUGUCACGU
263	ML.8.20	CGUUCGGUGGUGGACAGGGUAAUGUGGAGGGACCCGGUGAUUGUGUAUGU
264	ML.8.21	UGGAGGGUGCGGGAGAAAGUUUGAGGUCGGGUC'GUAUGAUGUGCGCUAG
265	ML.8.22	UCACGACGGAGGGCGGAUGAAGGGGGGAAGGUCUGAGUCCAUUGCCGUGU
266	ML.8.23	GGUUGGGCGUGAAUGGAGGGCAUUGUGUGGGUUUGUUGAGCCAGUUGGU
267	ML.8.24	ACGGGUCCCUAAGACGUCUGUUUCGAGAUGUGUGUUUCGGAAAGAGUAGCG

TABLE 16

Two point binding data for ligands from gag SELEX "A"

<u>Ligand</u>	% Ligand bound at 10 nM gag	% Ligand bound at 0.1 nM gag	% Ligand bound at 0.0 nM gag
SW8.1	30	6.6	3.2
SW8.2	7.3	2.9	1.9
SW8.4	27	2	1.3
SW8.6	4.1	1.7	1.5
SW8.20	16	4.1	4.7
SW8.24	20	4.5	2.6
SW8.25	11	1.1	1.0
SW8.27	32	4.6	5.3
SW8.30	3.7	0.8	0.8
SW8.31	6.3	2.4	2.7
SW8.37	4.2	1.8	1.0
SW8.40	15	8.5	3.8
SW10.7	8.6	3.5	3.0
SW10.12	11.2	0.6	0.6
SW10.22	33	3.1	1.7
SW10.25	19	10	13
SW10.28	33	31	16
SW10.34	11.8	7.9	8.2
SW10.39	17	0.8	0.6
SW10.43	6.7	4.1	3.0
Ψ-456	87	13.4	14.7
Round 0 RNA	3.5	4.0	6.0

Table 17. Affinity of nucleic acid ligands for the HIV-1 gag protein.

<u>Sequence ID no.</u>	<u>Ligand name</u>	<u>Kd for GST-gag</u>
231	SW8.4	1 nM
234	SW8.24	5 nM
235	SW8.25	5 nM
236	SW8.27	3 nM
238	SW8.31	41 nM
239	SW8.37	50 nM
242	SW10.12	30 nM
245	SW10.28	8 nM
255	ML8.7	12 nM
258	ML8.14	13 nM
263	ML8.20	10 nM
265	ML8.22	10 nM
268	ML6.1	2 nM
271	ML6.6	11 nM
272	ML6.8	3 nM
273	ML6.9	7 nM
276	ML6.17	4 nM

Table 18. Binding specificity of HIV-1 gag nucleic acid ligands

Sequence ID no.	Ligand Name	Binds to ^a	Frequency ^b
230	SW8.2	matrix	1
231	SW8.4	nucleocapsid	12
232	SW8.6	matrix	1
234	SW8.24	nucleocapsid	2
235	SW8.25	nucleocapsid	1
236	SW8.27	matrix	3
237	SW8.30	matrix	1
238	SW8.31	nucleocapsid	1
239	SW8.37	nucleocapsid	1
242	SW10.12	matrix	1
243	SW10.22	nucleocapsid	1
245	SW10.28	nucleocapsid	1
247	SW10.39	nucleocapsid	1
255	ML8.7	matrix	7
256	ML8.11	GST	1
257	ML8.12	filter	1
258	ML8.14	matrix	2
259	ML8.16	filter	1
260	ML8.17	filter	1
261	ML8.18	filter	1
262	ML8.19	filter	1
263	ML8.20	nucleocapsid	2
264	ML8.21	filter	1
265	ML8.22	nucleocapsid	1
266	ML8.23	filter	1
267	ML8.24	filter	1

Table 18 (page 2)

Sequence ID no.	Ligand Name	Sequence	Binds to	Frequency ^b
268	ML.6.1	ACAGCGAUUUUGUGUCGUCGCGGCUUUUGCAGAUUGGAUUGGAACCAUUGC	nucleocapsid	1
269	ML.6.2	GGGCUUGGGGUGGGGUCUUCGACGCGAUUUGGAUUGGAUUGCUUGCUGGCU	GST-gag	1
270	ML.6.3	AUUGGGAACGUGGCGCAUCGGGAGUGUAUGGUGGCUUGUGGCGGGUUA	GST-gag	1
271	ML.6.6	AGCCAGGUCUUCAAUGGUAGUUGUAGUUGGUGGUGCUUUGCAGAUUGAU	nucleocapsid	1
272	ML.6.8	UCGGUUAUGGAGAGCGGUAAGAGAGCGAGUAGCAAGUAACGGUGCAAAAG	matrix	1
273	ML.6.9	CCGACUUCGAUUGCAUUGUCAGGAGAGCGGGUGCAGAUUACGGGUUUG	nucleocapsid	1
274	ML.6.12	AGACUUGGUACGCGUGGUUAGCUAGGAGGGGGGAGAGAUUAGGCGAUAG	GST-gag	1
275	ML.6.16	GAGUUUUGCUUCGACAUUGGGUUAAGCUGGAGCAGGGUGACCUUGGACC	GST-gag	1
276	ML.6.17	UGGAGUGGGUAGCCGUGGGAGGUUUGGCAUCUUGUGUGGUGCCAAAGUUGU	?	1

^a Filter means the nucleic acid ligand binds to nitrocellulose filters and not to the GST-p55 (HIV-1 gag) protein used in the SELEX procedure. GST-gag means the nucleic acid ligand binds to the GST-p55 (HIV-1 gag) protein used in the SELEX procedure, but that binding to individual components of the HIV-1 gag polypeptide has not been determined. Nucleocapsid, matrix, and GST mean the nucleic acid ligand binds to the GST-p7 (HIV-1 nucleocapsid) protein, the HIV-1 p17 matrix protein, or the glutathione S-transferase protein described in Materials and Methods, respectively. A question mark indicates the nucleic acid ligand binds to the GST-p55 (HIV-1 gag) protein, but does not bind to GST, GST-p7 (HIV-1 nucleocapsid), GST-p15 (HIV-1 nucleocapsid), p17 HIV-1 matrix, or p24 HIV-1 capsid proteins.

^b Frequency indicates the number of clones sequenced for each nucleic acid ligand at the end of the SELEX procedure.

Table 19. Boundaries of HIV-1 gag nucleic acid ligands.

[illegible]

• A NC suffix indicates a HIV-1 nucleocapsid nucleic acid ligand. A MA suffix indicates a HIV-1 matrix nucleic acid ligand.

^b 5' indicates the position of the 5' nucleic acid ligand boundary. 3' indicates the position of the 3' nucleic acid ligand boundary. Underlined regions designate the minimal nucleic acid ligand sequence that is predicted to bind to the HIV-1 gag protein, based on the position of the 5' and 3' boundaries. Lower case lettering indicates a palindromic motif found in nucleocapsid ligands. The sequences of the fixed regions used in SELEX procedure "A" and "B" are indicated. The sequences evolved during the SELEX procedure are located between the fixed sequences.

Table 20. Binding properties of truncated HIV-1 gag nucleic acid ligands.

Sequence ID no.	Ligand Name ^a	Sequence of truncated nucleic acid ligand ^b	Binding ^c
277	ML6.1 _{NC} t13-63	gggUCAUACACAAGAACAGUGAUUUGUGUCUGCGGCUUUGCGAGUGUAU	+
278	ML6.6 _{NC} t15-74	gggUAUACACAAGAACAGGUCUUAAGUGUAGUUGUUAUUGGUGGUCUUGCAGAGUAU	+
279	ML6.8 _{NC} t33-82	gggAGAGGCGGUAGAGAGCGAGUAGCAAGUAAACGGUGCAAAAAGGCUCCGCC	+
280	ML8.7 _{NC} t40-72	GggUUUGGUUAGUCUGUGGAGGGGCCAUGAGGGGCUGGA	-
281	ML8.20 _{NC} t14-73	gggCAUACACAAGACGUCUGUGUGGACAGGGUAAUGUGGAGGGACCGGGUGAUUGUGUAUGUG	+
282	ML8.22 _{NC} t23-79	gggCAUACACAAGACGUCUGUGGAGCGGUAAGGGGGGAAGGUCGUGAGUCCAUUGCGUGCUCC	+
283	SW8.4 _{NC} t41-55	gggCGUGGUGCAUCUGUGccc	-
284	SW8.4 _{NC} t30-53	GggagCUACAGGGGAACCGUGGUGCAUCUG	+
285	SW10.12 _{NC} t28-56	gggGUUAUCAGCACACCGGUACAAAGAGGAUGC	-

^a The name of the full length nucleic acid ligand from which the truncate is derived is indicated, beginning with the letters ML or SW. The NC suffix indicates a truncation of the indicated HIV-1 nucleocapsid nucleic acid ligand. The MA suffix indicates a truncation of the indicated HIV-1 matrix nucleic acid nucleic acid ligand. The lower case "t" designates the RNA as a truncated nucleic acid ligand. The numbers following the "t" indicate the nucleotide positions from the full length nucleic acid ligand that are included in the truncated nucleic acid ligand.

^b Lower case letters are nucleotides added to facilitate generation of the truncated nucleic acid ligand by transcription or, in the case of SW8.4 to promote stem/loop formation, but are not found in the full length nucleic acid ligand sequence.

^c A plus indicates the truncated nucleic acid ligand binds to the appropriate HIV-1 protein at least 10 fold better than random RNA. A minus indicates the truncated nucleic acid ligand does not bind to the appropriate HIV-1 protein any better than random RNA.

Table 21. Binding properties of mutant HIV-1 gag nucleic acid ligands.

Sequence ID no. Binding ^a	Ligand Name ^a	Sequence of mutant nucleic acid ligand
286	ML6.8 _{MA} (D56-57)	GGGAAAAGCCGAAUCAUACACAAGAUCGGGUUAUGGAGAGAGCGGUGUAGAGAGGUAAGUAAACGGUGCAAAAGGCCUCCGCCAGAGACCAACCCGAGAA
287	SW8.4 _{NC} (G47C)	GGGAGACAAGAAUAAACGCCUCAACGUCUUCUACAGGGAAACCGUGGUCCAUUCUGUGAAAGUUGUAGAUUCCUAGUUUGGACAGGAGGCUCACACACAGGC
288	SW8.4 _{NC}	GGGAGACAAGAAUAAACGCCUCAACGUCUUCUACAGGGAAACCGUGGCGCGCCUGUGAGAGUUGUAGAUUCCUAGUUUGGACAGGAGGCUCACACACAGGC
		(GUCCAU45-50GCGCGC)

^a The name of the nucleic acid ligand that was mutated is indicated. The NC suffix indicates a mutant of the indicated HIV-1 nucleocapsid nucleic acid ligand. The MA suffix indicates a mutant of the indicated HIV-1 matrix nucleic acid ligand. The numbers and letters in parentheses following the nucleic acid ligand name indicate which nucleotides were changed, what their positions are, and what sequence they were changed to within the mutated nucleic acid ligand. Further details are described in the text. A "D" sign indicates nucleotides that were deleted.

^b A minus indicates the mutated nucleic acid ligand does not bind to the appropriate HIV-1 protein any better than random RNA.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Larry Gold
David Parma
Nebojsa Janjic
Michael Lochrie
- (ii) TITLE OF INVENTION: High-Affinity Nucleic Acid Ligands
To Secretory Phospholipase A₂ (sPLA₂),
Vascular Endothelial Growth Factor
(VEGF), and Human Immunodeficiency
Virus type-1 GAG (HIV-1 GAG)
- (iii) NUMBER OF SEQUENCES: 282
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Swanson & Bratschun, L.L.C.
 - (B) STREET: 8400 E. Prentice Avenue, Suite 200
 - (C) CITY: Englewood
 - (D) STATE: Colorado
 - (E) COUNTRY: USA
 - (F) ZIP: 80111
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 MB storage
 - (B) COMPUTER: IBM compatible
 - (C) OPERATING SYSTEM: MS-DOS
 - (D) SOFTWARE: WordPerfect 6.0
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT/US96/02942
 - (B) FILING DATE: 04 MAR 96
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/447,169
 - (B) FILING DATE: 19 MAY 1995
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/447,172
 - (B) FILING DATE: 19 MAY 1995
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/399,412
 - (B) FILING DATE: 06 MARCH 1995
- (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/233,012
(B) FILING DATE: 25 APRIL 1994

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/205,515
(B) FILING DATE: 03 MARCH 1994

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/117,991
(B) FILING DATE: 08 SEPTEMBER 1993

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/964,624
(B) FILING DATE: 21 OCTOBER 1992

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/931,473
(B) FILING DATE: 17 AUGUST 1992

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/714,131
(B) FILING DATE: 10 JUNE 1991

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/536,428
(B) FILING DATE: 11 JUNE 1991

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Barry J. Swanson
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(A) TELEPHONE: (303) 793-3333
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGAGCUCAG AAUAAACGCU CAANNNNNNN NNNNNNNNNN NNNNNNNNNN
NNNUUCGACA UGAGGCCCGG AUCCGGC

50

77

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGAAGCTTA ATACGACTCT ATAGGGAGCT CAGAATAAAC GCTCAA

46

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCCGGATCCG GGCCTCATGT CGAA

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGGAGGACGA UGCGGNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN

50

NNNNNNNNNN NNNNNCAGAC GACUCGCCCC A

81

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TAATACGACT CACTATAGGG AGGACGATGC GG

32

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
TCGGGCGAGT CGTCCTG 17
- (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ix) FEATURE:
- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
- (ix) FEATURE:
- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
- GGGAAAAGCG AAUCAUACAC AAGANNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN NNNNNNNNNN NNNNGCUCCG CCAGAGACCA ACCGAGAA 98
- (2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
- TAATACGACT CACTATAGGG AAAAGCGAAT CATAACAAG A 41
- (2) INFORMATION FOR SEQ ID NO:9:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
- TTCTCGGTTG GTCTCTGGCG GAGC 24
- (2) INFORMATION FOR SEQ ID NO:10:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
- GGGAGCUCAG AAUAAACGCU CAAUCUCAUG CUCGUCGCAC GGCGUAACCU 50
AUUUCGACAU GAGGCCCGGA UCCGGC 76

- (2) INFORMATION FOR SEQ ID NO:11:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
- GGGAGCUCAG AAUAAACGCU CAAUCUCAUU GCUCGUCGCA CGGCGUAACC 50
UAUUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:12:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
- GGGAGCUCAG AAUAAACGCU CAAAGCUCAU CGUCUCGCAA GGCGUAUCCU 50
AUUUCGACAU GAGGCCCGGA UCCGGC 76
- (2) INFORMATION FOR SEQ ID NO:13:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
- GGGAGCUCAG AAUAAACGCU CAAACCUCGC CUAUGUUCGC GCGGCGUAUC 50
CUAUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:14:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
- GGGAGCUCAG AAUAAACGCU CAACAGCCAA UGUGUCCCGU ACGGCGUAUC 50
CUAUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:15:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
 GGGAGCUCAG AAUAAACGCU CAACGUGGA UACCAUCGCA CGGCGUAUCC 50
 UGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
 GGGAGCUCAG AAUAAACGCU CAAUUGCAU CAUGUACCGC AAGACGUAU 50
 CUAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
 GGGAGCUCAG AAUAAACGCU CAAUUGCAU CAUGUACCGC AAGACGUAU 50
 CUAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
 GGGAGCUCAG AAUAAACGCU CAAUUGCAU CAUGUACCGU AAGACGUAU 50
 CUAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 78 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
 GGGAGCUCAG AAUAAACGCU CAAUUGCAU CAUGUACCGC AAGACGUAU 50
 CUAUUCGAC AUGAGGCCCG GAUCCGGC 78

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCC CGGCUGAGAC 50
 ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCC CGGCUGAGAC 50
 ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGUU CGGCUGAGAC 50
 ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGGAGCUCAG AAUAAACGCU CAAUGCCUCU GCUUACGGGU AAUGCCGAGA 50
 CACUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
GGGAGCUCAG AAUAAACGCU CAAAGUCCUC UCCUUACGGU UCGCCCGAGA 50
UAAUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCC CGGCUGAGAC 50
AUUUCGACAU GAGGCCCGGA UCCGGC 76
- (2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCU CGGCUGAGAC 50
ACUUCGACAU GAGGCCCGGA UCCGGC 76
- (2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGUC CGGCUGAGAC 50
ACUUCGACAU GAGGCCCGGA UCCGGC 76
- (2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCC CGGCUGAGAC 50
GCUUCGACAU GAGGCCCGGA UCCGGC 76

- (2) INFORMATION FOR SEQ ID NO:29:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
- GGGAGCUCAG AAUAAACGCU CAAGGCCUCU GCUUACGGCU AAUGCCGAGA 50
- CGCUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:30:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
- GGGAGCUCAG AAUAAACGCU CAAGANCUCU GCUUACAGCC CGGCUGGGAC 50
- ACUUCGACAU GAGGCCCGGA UCCGGC 76
- (2) INFORMATION FOR SEQ ID NO:31:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
- GGGAGCUCAG AAUAAACGCU CAACUACAG UUCGGCUGAG AGAAGACGCA 50
- UACUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:32:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ix) FEATURE:
- (D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine
- (ix) FEATURE:
- (D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
- GGGAGCUCAG AAUAAACGCU CAACUACAG UUCGGCUGAG AGAAGACGCA 50
- UACUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:33:
- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GGGAGCUCAG AAUAAACGCU CAACUUACAG UUCGGCUGAG ACGAAGAUCG 50
ACCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGAGCUCAG AAUAAACGCU CAACUUACAG GAGAUUCCAU CUCGCUGAGA 50
CGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGGAGCUCAG AAUAAACGCU CAACUUACGG CAGCGAUUGC UGGCCGAGAA 50
ACCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGGAGCUCAG AAUAAACGCU CAACUUACGG GUAAAGCCGA GAAAUGUUAU 50
UGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine

(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
GGGAGCUCAG AAUAAACGCU CAACUUACGG GUAAAGCCGA GAAAUGUUAU 50
UGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:
GGGAGCUCAG AAUAAACGCU CAAUGUCUUU ACAGGUAAAG CUGAGGAAUC 50
GUUUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:
GGGAGCUCAG AAUAAACGCU CAAUGUCUUA CGGGUAAAGC CGAGAAAGUU 50
UCCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:
GGGAGCUCAG AAUAAACGCU CAAGGCUGGG UCUUUUACAG GUAAAGCUGA 50
GAAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO: 41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
GGGAGCUCAG AAUAAACGCU CAAAGUCUUA CGGGUAAAGC CGAGAAAGUU 50
UCCUUCGACA UGAGGCCCGG AUCCGGC 77

- (2) INFORMATION FOR SEQ ID NO:42:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 76 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
- GGGAGCUCAG AAUAAACGCU CAAUCAUGUC AUUACGGGUA AAGCCGAGUU 50
- UCUUCGACAU GAGGCCCGGA UCCGGC 76
- (2) INFORMATION FOR SEQ ID NO:43:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:
- GGGAGCUCAG AAUAAACGCU CAAAUCAUGU CAUACGGGU AAAGCCGAGU 50
- UUCUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:44:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
- GGGAGCUCAG AAUAAACGCU CAACACGAGG GUGGGUGGGU GGCCGAGCGC 50
- UUGUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:45:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
- GGGAGCUCAG AAUAAACGCU CAACACGGGG GUGGGUGGGU GGCCGAGCGC 50
- UUGUUCGACA UGAGGCCCGG AUCCGGC 77
- (2) INFORMATION FOR SEQ ID NO:46:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:
GGGAGCUCAG AAUAAACGCU CAAUGCCUCA UGCCAAUGUG GGAGGGUGGG 50
UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:
GGGAGCUCAG AAUAAACGCU CAACGCCUCA UGCCAACGCG GGAGGGUGGG 50
UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:
GGGAGCUCAG AAUAAACGCU CAACGCCUCA UGCCAACGUG GGAGCGUGGG 50
UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:
GGGAGCUCAG AAUAAACGCU CAACGCCUCA UGCCAAUGCG GGAGGGUGGG 50
UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:50:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine

(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:
GGGAGCUCAG AAUAAACGCU CAACGCCUCA UGCCAAUGCG GGAGGGUGGG 50

UGGUUCGACA UGAGGCCCGG AUCCGGC

77

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GGGAGCUCAG AAUAAACGCU CAAUCCGGGA GCUGAAAAAC AUGCCGUUAG 50
CCGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GGGAGCUCAG AAUAAACGCU CAAUCCGGGA GCUGAAAAAC AUGCCGUUAG 50
CCAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC AGUGUCCAGC 50
AUCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:
GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:56:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:
GGGAGCUCAG AAUAAACGCU CAAGCCGAAC CGAAUGGAGG UGAGGGGAUU 50
GCGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:57:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:
GGGAGCUCAG AAUAAACGCU CAAGACCACG UCCGAACGAA CACCGCCACG 50
CAUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:58:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:
GGGAGCUCAG AAUAAACGCU CAACCAACGA CACUCACGCA UUGCCCACGA 50
ACGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:59:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:
 GGGAGCUCAG AAUAAACGCU CAAACAACGG CCCACACGGG AGAUCCGAGA 50
 AAAGUUCGAC AUGAGGCCCG GAUCCGGC 78

(2) INFORMATION FOR SEQ ID NO:60:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 99 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:
 GGGAAAAGCG AAUCAUACAC AAGACCGGCC GGGGAAACCC GAGGUCCGAG 50
 GUAACGCAUG GCGCCUCACC GAGUCGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:61:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 99 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:
 GGGAAAAGCG AAUCAUACAC AAGACCGGCC GGGGAAACCC GAGGUCCGAU 50
 GUAACGCAUG GCGCCUCACC GAGUCGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:62:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 99 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:
 GGGAAAAGCG AAUCAUACAC AAGACCGGCC GGGGAAACCC GAGAUCCGAG 50
 GUAACGCAUG GCGCCUCACC GAGUCGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC GCGCCAUAG CCGAGAUCCG	50
AGGUUGUACG AUGACAACUC AGUGCUCGCG CAGAGACCAA CCGAGAA	97

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC GCGCCAUAG CCGAGAUCCG	50
AGGUGUUGAA CGAUAAUCG GUGCUCGCGC AGAGACCAAC CGAGAA	96

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GGGAAAAGCG AAUCAUACAC AAGAGACCGG CCAGCCAAGG CGCUGAGAU	50
CGAGGUUCA GAACCAUCG GGUUGGCUCC GCCAGAGACC AACCGAGAA	99

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:
 GGGAAAAGCG AAUCAUACAC AAGACCGGCC CGGUAUGUAG CCGGAGAUCC 50
 GAGACUUGCU GAACGAGGUG CCACGGCUCC GCCAGAGACC AACCGAGAA 99
- (2) INFORMATION FOR SEQ ID NO:67:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 99 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:
 GGGAAAAGCG AAUCAUACAC AAGACCGGCC CGGUGUGUAG CCGGAGAUCC 50
 GAGACUUGCU GAACGAGGUG CCACGGCUCC GCCAGAGACC AACCGAGAA 99
- (2) INFORMATION FOR SEQ ID NO:68:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 99 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:
 GGGAAAAGCG AAUCAUACAC AAGACCGGCC CGGUGUGCAG CCGGAGAUCC 50
 GAGACUUGCU GAACAAGGUG CCACGGCUCC GCCAGAGACC AACCGAGAA 99
- (2) INFORMATION FOR SEQ ID NO:69:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 99 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ix) FEATURE:
 (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC CCGCCAAUCA AGGGAGAUCC	50
GAGGAAUUGG AAUGUUUGUG AGUGAGCUCC GCCAGAGACC AACCGAGAA	99

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GGGAAAAGCG AAUCAUACAC AAGAGUGUGC GGUGCAUGCG UGGUGAAAGG	50
GGGUGGGGGA AGAAAAACCG GCCCGCUCCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GGGAAAAGCG AAUCAUACAC AAGAGUGUGC GGUGCAUGCG UGGUGAAAGG	50
UGGGUUGUGG AGGAAGACCG UGCCGCUCCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAUGCGGCG AGUGGAGGGG	50
--	----

GACUGAUGGA GGGCGAGACC GUGUGCUCGG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAGGCGGCG AGUGGAGGAG 50

GACUGAUGGA GGGCGAGACC GCAGGCUCGG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAAGCGGCG AGUGGAGGAG 50

GACUGAUGGA GGGCGAGACC GCAAGCUCGG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAUGCGGCG AGUGGAGGAG 50

GACUGAUGGA GGGCGAGACC GUGUGCUCGG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
- (ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:
- | | |
|--|----|
| GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAGGCGGCG AGUGGAGGAG | 50 |
| GACUGAUGGA GGGCGAGACC GCGUGCUCG CCAGAGACCA ACCGAGAA | 98 |
- (2) INFORMATION FOR SEQ ID NO:77:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
- (ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:
- | | |
|--|----|
| GGGAAAAGCG AAUCAUACAC AAGACCCCUU GAGCUCGUGA CGCAGGAGGA | 50 |
| GGGCCGAGGA GGAAAGUCGU CACAGCUCG CCAGAGACCA ACCGAGAA | 98 |
- (2) INFORMATION FOR SEQ ID NO:78:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
- (ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:
- | | |
|--|----|
| GGGAAAAGCG AAUCAUACAC AAGAGGAUGG CGGCAAGGCG CGAAAGGGAG | 50 |
| GAUCGAGGAG GAAUCGCGUC AGGAGCUCG CCAGAGACCA ACCGAGAA | 98 |
- (2) INFORMATION FOR SEQ ID NO:79:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GGGAAAAGCG AAUCAUACAC AAGAGCCAGC GAGUGUCGAC AGUGUGGGUG	50
GAAGUGACGG GAGGAUUGGA GACGC ⁰ CCGC CAGAGACCAA CCGAGAA	97

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GGGAAAAGCG AAUCAUACAC AAGACUGGUU GUGCGGACCC AGUGAGUGGG	50
AGGACGUGAG GGUGGCAGCU GGGC ⁰ CCGCC AGAGACCAAC CGAGAA	96

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GGGAGGACGA UGCGGAGGGU GGAUCGUGGA GGAAAAGCAU CGUGUGUAAC	50
CGAACCGAUC GUGGYCAGAC GACUCGCCCG A	81

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:
GGGAGGACGA UGCGGGUAGG GAUGAAGUGC GAUGUGAAUC CGGGUGCUAG 50
CGAUGAUGUG UGCCCCAGAC GACUCGCCCCG A 81

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:
GGGAGGACGA UGCGGGUAGG GAGACAGACA CACACGCGGA AAGUAGAGCC 50
AUCGUAACAU GCCCCAGAC GACUCGCCCCG A 81

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:
GGGAGGACGA UGCGGGUAGG GAUAAGCGAG UGUACAGCGA AUACGACUCG 50
GAAUGCUUGG UGCGCCAGAC GACUCGCCCCG A 81

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:
GGGAGGACGA UGCGGUGAAA GAGAAAGGUU GAGAUGAUUA CAAGCGAAUU 50
GGAUAAAGUGU CUGGCCAGAC GACUCGCCCCG A 81

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GGGAGGACGA UGCGGUGAAA UGAGAAAUGG AUUGAUGAUG AUUACAAGAG	50
AAUUGGAUAA GUGUCUGGUC AGACGACUCG CCCGA	85

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GGGAGGACGA UGCGGGAGGG AAGGGUGGAA CGGAACUCCG AUAAAGCUGU	50
ACAAGUACGU GGGGUCAGAC GACUCGCCCG A	81

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GGGAGGACGA UGCGGAUAAG GAGGAGCAAG CGAGAAAUUG AGAAGUAACA	50
AGAUCGACAU GGCCCCAGAC GACUCGCCCG A	81

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ix) FEATURE:
 - (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
- (ix) FEATURE:
 - (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GGGAGGACGA UGCGGAUAAG GAUAAGAUCG AACGAGAGUG AACAAAGUUA	50
AAUACAGUCU GGGGCAGAC GACUCGCCCCG A	81

- (2) INFORMATION FOR SEQ ID NO:90:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 81 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GGGAGGACGA UGCGGGCUAA GGGAAGACAA UGAGAUAGCA GACAAUCAAC	50
UACACCCAUG UGCGUCAGAC GACUCGCCCCG A	81

- (2) INFORMATION FOR SEQ ID NO:91:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 81 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

GGGAGGACGA UGCGGUGAGC UUAGGAUAGG AGCAACAAGU AGAGUAGAGU	50
GAUAACUAGG GUGGCCAGAC GACUCGCCCCG A	81

- (2) INFORMATION FOR SEQ ID NO:92:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 81 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GGGAGGACGA UGCGGUGACA AAUGAGCAAG UAGCGAUAGA UGUGAUGGAC 50
 AGAGACAGCC GGGGCCAGAC GACUCGCCCCG A 81

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GGGAGGACGA UGCGGAAAUG UGUUAGUGAA UGAUUGAGAG AAGAUAGAUG 50
 AUGUUGAAGU CUGGCCAGAC GACUCGCCCCG A 81

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

GGGAGGACGA UGCGGAACUA AAAGACAGAG AGAAAACGAC AAUACGAAGU 50
 ACAUAUAACC CUGGCCAGAC GACUCGCCCCG A 81

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GGGAGGACGA UGCGGAAGUA GAUGAUGGAU UGAGAUGUAA GUGUCAGUAU 50

GAAGAGUCUC UGGGCCAGAC GACUCGCCCG A

81

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GGGAGGACGA UGCGGAGGAA AUGAAGUAGU GAGAGUAUAA CAUGAUUAUG 50

AAUACGUGAU GUGGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

YMUCAUGUHY CGYAMGRCGU AUYCUAU 27

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

CUCWRCUUAC RGBYMVGCYGA 23

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

MAYGNNGGWWG GGUGGGUGG 19

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs

- (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂, cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂, uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:
RAGACCGGCC NGSNNNNNNN SCNGAGAUCC GAGG 34
- (2) INFORMATION FOR SEQ ID NO:101:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:
CUCAUUGCUU GUCGACGGC GUAACCUAUU UCGACAUGA 39
- (2) INFORMATION FOR SEQ ID NO:102:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:
GGUCUCAUUG CUCGUCGCAC GGC GUAACCU AUUUGACAUG GAG 43
- (2) INFORMATION FOR SEQ ID NO:103:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:
AGACCUCUGC UUACAGCCCG GCUGAGACAC UUCGACAUGA GG 42
- (2) INFORMATION FOR SEQ ID NO:104:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:
UAAACGCUCA ACUUACAGUU CGG CUGAGAC GAAGAU CGAC CUUCGACA 48

- (2) INFORMATION FOR SEQ ID NO:105:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 48 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:
- GGGAAGACCU CUGCUUACAG CCCGGCUGAG ACACUUCGAC AUGAGGCC 48
- (2) INFORMATION FOR SEQ ID NO:106:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:
- GGGAUAAACG CUCAACUAC AGUUCGGCUG AGACGAAGAU CGACCUUCGA 50
CAU 53
- (2) INFORMATION FOR SEQ ID NO:107:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:
- GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC AGUGUCCAGC 50
AUCUUCGAC 59
- (2) INFORMATION FOR SEQ ID NO:108:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 59 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:
- GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGAC 59
- (2) INFORMATION FOR SEQ ID NO:109:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 62 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGACA UG 62

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 61 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

GGAGCUCAGA AUAACGCU AAGCUCUGG AUGAUGCCA CUGUCCAGCA 50
UCUUCGACAU G 61

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 55 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

GGGAGCUAUA AACGCAGCUC UGGAUGAUG CCCACUGUCC AGCAUCUUCG 50
ACAUG 55

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 83 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGACA UGUGUAGCUA AACAGCUUUA GGA 83

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂, cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂, uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

AAGACCGGCC GGGGAAACCC GAGGUCCGAG GUAACGCA 38

(2) INFORMATION FOR SEQ ID NO:114:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂, cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂, uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:
AAGACCGGCC GGCGCCAUAG CCGAGAUCCG AGGUGUUG 38

(2) INFORMATION FOR SEQ ID NO:115:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂, cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂, uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:
GAGACCGGCC AGCCAAGGCG CUGAGAUCCG AGGUUUA 38

(2) INFORMATION FOR SEQ ID NO:116:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂, cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂, uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:
AAGACCGGCC CGGUGUGCAG CCGGAGAUCC GAGACUUGCU G 41

(2) INFORMATION FOR SEQ ID NO:117:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:
GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGA 47
- (2) INFORMATION FOR SEQ ID NO:118:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:
GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGAACG 50
AUAGACCAAC CGAGAA 66
- (2) INFORMATION FOR SEQ ID NO:119:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:
GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGAGAC 50
CAACCGAGAA 60
- (2) INFORMATION FOR SEQ ID NO:120:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:
GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGAACG 50

AUCCGAGAA

59

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGCCGA 50
GAA 53

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL C'S ARE 2'-NH₂ cytosine

(ix) FEATURE:

- (D) OTHER INFORMATION: ALL U'S ARE 2'-NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

GGGAGGACGA UGCGGUAGUA ACGGAUACUG AUCCGAGGUU AUAGUCACUA 50
UAUCAUCCGC UGCGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

GGGAGCUCAG AAUAAACGCU CAANNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNUCCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:
CCGAAGCTTA ATACGACTCA CTATAGGGAG CTCAGAATAA ACGCTCAA 48

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:
GCCGGATCCG GGCCTCATGT CGAA 24

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 2-4, 15, 17-18
- (D) OTHER INFORMATION: C at positions 2-4, 15,
17-18 are 2'NH₂ cytosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 5, 8, 11, 23
- (D) OTHER INFORMATION: U at positions 5, 8, 11,
23 are 2'NH₂ uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:
ACCCUGAUGG UAGACGCCGG GGUG 24

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 2-4, 15, 17-18
- (D) OTHER INFORMATION: C at positions 2-4, 15,
17-18 are 2'NH₂ cytosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 5, 8, 11, 23
- (D) OTHER INFORMATION: U at positions 5, 8, 11

and 23 are 2'NH₂ uracil

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 12, 14
- (D) OTHER INFORMATION: A at positions 12 and

14 are 2'OMe adenosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 13, 16, 19-21, 22, 24
- (D) OTHER INFORMATION: G at positions 13, 16,

19-21, 22 and 24 are 2'OMe guanosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

ACCCUGAUGG UAGACGCCGG GGUG

24

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 6-8, 19, 21-22
- (D) OTHER INFORMATION: C at positions 6-8,

19 and 21-22 are 2'NH₂ cytosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 9, 12, 15, 27
- (D) OTHER INFORMATION: U at positions 9, 12, 15

and 27 are 2'NH₂ uracil

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 1-4, 29-33
- (D) OTHER INFORMATION: T at positions 1-4

and 29-33 are 2' deoxy phosphorothioate thymidine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

TTTACCCUG AUGGUAGACG CCGGGUGTT TTT

33

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: All A's, C's, G's,

T's are 2' deoxy-nucleotide derivatives

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 1-4, 29-33
- (D) OTHER INFORMATION: T at positions 1-4

and 29-33 are phosphorothioate thymidine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

TTTTACCCTG ATGGTAGACG TTGGGGTGTT TTT

33

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (D) OTHER INFORMATION: All A's, C's, G's,

U's are 2'OMe-nucleotide derivatives

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 1-4, 29-33
- (D) OTHER INFORMATION: T's at positions

1-4 and 29-33 are 2' deoxy phosphorothioate thymidine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

TTTTACCCUG AUGGUAGACG CCGGGGUGTT TTT

33

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 6-8, 19, 21-22
- (D) OTHER INFORMATION: C at positions 6-8,

19 and 21-22 are 2'NH₂ cytosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 9, 12, 15, 27
- (D) OTHER INFORMATION: U at positions 9, 12,

15 and 27 are 2'NH₂ uracil

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 5, 16
- (D) OTHER INFORMATION: A at positions 5 and

16 are 2'OMe adenine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 13, 17, 20, 23-26, 28

(D) OTHER INFORMATION: G at positions 13, 17,

20, 23-26 and 28 are 2'OMe guanosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 1-4, 29-33

(D) OTHER INFORMATION: T's at positions 1-4

and 29-33 are 2' deoxy phosphorothioate thymidine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

TTTACCCUG AUGGUAGACG CCGGGGUGTT TTT

33

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 6-8, 19, 21-22

(D) OTHER INFORMATION: C at positions 6-8,

19 and 21-22 are 2'NH₂ cytosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 5, 8, 11, 23

(D) OTHER INFORMATION: U at positions 9, 12,

15 and 27 are 2'NH₂ uracil

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 5, 16

(D) OTHER INFORMATION: A at positions 5 and

16 are 2'OMe adenine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 13, 17, 20, 24-26, 28

(D) OTHER INFORMATION: G at positions 13, 17,

20, 24-26 and 28 are 2'OMe guanosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 1-4, 29-33

(D) OTHER INFORMATION: T's at positions 1-4

and 29-33 are 2' deoxy phosphorothioate thymidine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

TTTTACCCUG AUGGUAGACG CCGGGGUGTT TTT

33

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 6-8, 19, 21-22
- (D) OTHER INFORMATION: C at positions 6-8, 19 and 21-22 are 2'NH₂ cytosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 9, 12, 15, 27
- (D) OTHER INFORMATION: U at positions 9, 12, 15 and 27 are 2'NH₂ uracil

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 5, 16
- (D) OTHER INFORMATION: A at positions 5 and 16 are 2'OMe adenine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 13, 17, 20, 23-26, 28
- (D) OTHER INFORMATION: G at positions 13, 17, 20, 23-26 and 28 are 2'OMe guanosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 11
- (D) OTHER INFORMATION: A at position 11 is phosphorothioate adenine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 1-4, 29-33
- (D) OTHER INFORMATION: T's at positions 1-4 and 29-33 are 2' deoxy phosphorothioate thymidine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

TTTTACCCUG AUGGUAGACG CCGGGGUGTT TTT

33

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 2-4, 15, 17-18

(D) OTHER INFORMATION: C at positions 2-4, 15 and 17-18 are 2'NH₂ cytosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 5, 8, 11, 23

(D) OTHER INFORMATION: U at positions 5, 8, 11 and 23 are 2'NH₂ uracil

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 9

(D) OTHER INFORMATION: G at position 9 is a 2:1 mixture of 2'OMe guanosine and 2'OH guanosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 12, 14

(D) OTHER INFORMATION: A at positions 12 and 14 are a 2:1 mixture of 2'OMe adenine and 2'OH adenine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

ACCCUGAUGG UAGACGCCGG GGUG

24

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 2-4, 15, 17-18

(D) OTHER INFORMATION: C at positions 2-4, 15 and 17-18 are 2'NH₂ cytosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 5, 8, 11, 23

(D) OTHER INFORMATION: U at positions 5, 8, 11 and 23 are 2'NH₂ uracil

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 1 and 7

(D) OTHER INFORMATION: A at positions 1 and 7 are a 2:1 mixture of 2'OMe adenine and 2'OH adenine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 19 and 21
- (D) OTHER INFORMATION: G at positions 19 and 21 are a 2:1 mixture of 2'OMe guanosine and 2'OH guanosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:
ACCCUGAUGG UAGACGCCGG GGUG

24

(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 2-4, 15, 17-18
- (D) OTHER INFORMATION: C at positions 2-4, 15 and 17-18 are 2'NH, cytosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 5, 8, 11, 23
- (D) OTHER INFORMATION: U at positions 5, 8, 11 and 23 are 2'NH, uracil

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 6, 20, 24
- (D) OTHER INFORMATION: G at positions 6, 20, and 24 are a 2:1 mixture of 2'OMe guanosine and 2'OH guanosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:
ACCCUGAUGG UAGACGCCGG GGUG

24

(2) INFORMATION FOR SEQ ID NO:137:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 2-4, 15, 17-18
- (D) OTHER INFORMATION: C at positions 2-4, 15 and 17-18 are 2'NH, cytosine

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 5, 8, 11, 23

(D) OTHER INFORMATION: U at positions 5, 8, 11 and 23 are 2'NH₂ uracil

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 10, 13, 16

(D) OTHER INFORMATION: G at positions 10, 13 and 16 are a 2:1 mixture of 2'OMe guanosine and 2'OH guanosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

ACCCUGAUGG UAGACGCCGG GGUG

24

(2) INFORMATION FOR SEQ ID NO:138:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

ATCCGCCTGA TTAGCGATAC TACAACGGCG TGGAAGACTA GAGTGCAGCC
GAACGCATCT AACTTGAGCA AAATCACCTG CAGGGG

50

86

(2) INFORMATION FOR SEQ ID NO:139:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

ATCCGCCTGA TTAGCGATAC TACGCTACAA GTCCGCTGTG GTAGACAAGA
GTGCAGGCAA GACTTGAGCA AAATCACCTG CAGGGG

50

86

(2) INFORMATION FOR SEQ ID NO:140:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

ATCCGCCTGA TTAGCGATAC TAGGCCCGTC GAAGNTAGAG CGCAGGGCCC
CAAAATACCG ACTTGAGCAA AATCACCTGC AGGGG

50

85

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:
- ATCCGCCTGA TTAGCGATAC TGTACCATCC ACGGTTTACG TGGACAAGAG 50
- GGCCCTGGTA CACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO:142:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:
- ATCCGCCTGA TTAGCGATAC TTCACTACAA GTCCGCCGTG GTAGACAAGA 50
- GTGCAGGCAA GACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO:143:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:
- ATCCGCCTGA TTAGCGATAC TACCGCTGTG TAGTTCCTTT AGGACTAGAG 50
- GGCCGCCTAC ACTTGAGCAA AATCACCTGC AGGGG 85
- (2) INFORMATION FOR SEQ ID NO:144:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:
- ATCCGCCTGA TTAGCGATAC TTAGGCTTGA CGTCTTCTAG ACTAGAGTGC 50
- AGTCAAACCC ACTTGAGCAA AATCACCTGC AGGGG 85
- (2) INFORMATION FOR SEQ ID NO:145:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:
- ATCCGCCTGA TTAGCGATAC TTGCAGGTCG ACTCTAGAGG ATCCCCGGGT 50
- ACCGAGCTCG AACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO:146:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:
ATCCGCCTGA TTAGCGATAC TACGGTTTAC GTGGACAAGA GGGCCCTGGT 50
ACACTTGAGC AAAATCACCT GCAGGGG 77
- (2) INFORMATION FOR SEQ ID NO:147:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:
ATCCGCCTGA TTAGCGATAC TGGTGGACTA GAGGNCAGCA AACGATCCTT 50
GGTTGCGGTC CACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO:148:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:
ATCCGCCTGA TTAGCGATAC TTCAAGCACT CCCGTCTTCC AGACAAGAGT 50
GCAGGGCCTC TACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO:149:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:
ATCCGCCTGA TTAGCGATAC TCGTGATGGA CAAGAGGGCC CTATCCACCG 50
GATATCCGTC ACTTGAGCAA AATCACCTGC AGGGG 85
- (2) INFORMATION FOR SEQ ID NO:150:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

ATCCGCCTGA TTAGCGATAC TCAAGCAGTG CCCGTCTTCC AGACAAGAGT 50
GCAGGCCTCT ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

ATCCGCCTGA TTAGCGATAC TTGATCCACC GTTTATAGTC CGTGGTAGAC 50
AAGAGTGCAG GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

ATCCGCCTGA TTAGCGATAC TAACACACAA GAGGACAGTT ACAGGTAACA 50
TCCGCTCAGG ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 83 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

ATCCGCCTGA TTAGCGATAC TAGTGGCGTC TATAGACAAG AGTGCAGCCC 50
GAGTTTCAAC TTGAGCAAAA TCACCTGCAG GGG 83

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 84 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

ATCCGCCTGA TTAGCGATAC TCCACAAGAG GGCAGCAAGT GTACAACTAC 50
AGCGTCCGGA CTTGAGCAAA ATCACCTGCA GGGG 84

(2) INFORMATION FOR SEQ ID NO:155:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

CTACCTACGA TCTGACTAGC GCAGGGCCAC GTCTATTTAG ACTAGAGTGC	50
AGTGGTTTCG TTA CTCTCAT GTAGTTCCT	79

(2) INFORMATION FOR SEQ ID NO:156:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

CTACCTACGA TCTGACTAGC ACGGTCCAAA GGTTCCTCCAT CCGTGACTA	50
GAGGGCACGT GCTTAGCTTA CTCTCATGTA GTTCCT	86

(2) INFORMATION FOR SEQ ID NO:157:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

CTACCTACGA TCTGACTAGC CCGTCGCGTG ACTATAACCA CACGCAGACT	50
AGAGTGCAGG GCTTAGCTTA CTCTCATGTA GTTCCT	86

(2) INFORMATION FOR SEQ ID NO:158:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 80 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

CTACCTACGA TCTGACTAGC CCGAATGGGG CTGCGACTGC AGTGGACGTC	50
ACGTCGTTAG CTTACTCTCA TGTAGTTCCT	80

(2) INFORMATION FOR SEQ ID NO:159:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 80 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

CTACCTACGA TCTGACTAGC ACGCAAGAGA GTCNCCGAAT GCAGTCTCAG	50
CCGCTAACAG CTTACTCTCA TGTAGTTCCT	80

(2) INFORMATION FOR SEQ ID NO:160:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 84 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

ATCCGCCTGA TTAGCGATAC TCANNCACT GCAAGCAATT GTGGCCCAAA	50
GGGCTGAGTA CTTGAGCAAA ATCACCTGCA GGGG	84

(2) INFORMATION FOR SEQ ID NO:161:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

ATCCGCCTGA TTAGCGATAC TGCTCGCTTA CAAAAGGGAG CCACTGTAGC	50
CCAGACTGGA CACTTGAGCA AAATCACCTG CAGGGG	86

(2) INFORMATION FOR SEQ ID NO:162:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

ATCCGCCTGA TTAGCGATAC TGGTTATGGT GTGGTTCCGA ATGGTGGGCA	50
AAGTAACGCT TACTTGAGCA AAATCACCTG CAGGGG	86

(2) INFORMATION FOR SEQ ID NO:163:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

ATCCGCCTGA TTAGCGATAC TGCTTGTCGC TCCGAAGGGG CGCGTATCCA	50
AGGACGGTTC ACTTGAGCAA AATCACCTGC AGGGG	85

(2) INFORMATION FOR SEQ ID NO:164:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 83 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:
ATCCGCCTGA TTAGCGATAC TTATGGAGTG GTTCCGAATG GTGGGCAAAG 50
TAACGCTTAC TTGAGCAAAA TCACCTGCAG GGG 83

(2) INFORMATION FOR SEQ ID NO:165:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:
CTACCTACGA TCTGACTAGC TGCNNGCGGG CGGTTCTCCG GATGGGACCA 50
TAAGGCTTTA GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:166:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 80 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:
CTACCTACGA TCTGACTAGC ACAAGGGGTC CTGNNGAATG GGGGAATACG 50
CTAGCCGAAG CTTACTCTCA TGTAGTTCCT 80

(2) INFORMATION FOR SEQ ID NO:167:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:
CTACCTACGA TCTGACTAGC AACACGAGCA TGTGGGGTCC CTCCGAATG 50
GGGGGTACAG GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:168:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:
CTACCTACGA TCTGACTAGC GAGGCATTAG GTCCGAATGG TAGTAATGCT 50
GTCGTGCCTT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:169:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 80 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:
 CTACCTACGA TCTGACTAGC CCGAATGGGG CTGCGACTGC AGTGGACGTC 50
 ACGTCGTTAG CTTACTCTCA TGTAGTTCCT 80

(2) INFORMATION FOR SEQ ID NO:170:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 86 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:
 CTACCTACGA TCTGACTAGC GAGGAGGTGC GTTGTCCGAA GGGGTCGTTA 50
 GTCACCTCGT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:171:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 81 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:
 CTACCTACGA TCTGACTAGC GCAAGGGGTC CTGCCGAATG GGGGAATACG 50
 CTAGCCGAAA GCTTACTCTC ATGTAGTTCC T 81

(2) INFORMATION FOR SEQ ID NO:172:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 71 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:
 CTACCTACGA TCTGACTAGC ATCCTTCCGA ATGGGGGAAA TGGCGNCCCA 50
 GCTTACTCTC ATGTAGTTCC T 71

(2) INFORMATION FOR SEQ ID NO:173:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 82 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:
 CTACCTACGA TCTGACTAGC ACGCAAGAGA GGTCNCCGAA TGGCAGTCTC 50

AGCCGCTAAC AGCTTACTCT CATGTAGTTC CT

82

(2) INFORMATION FOR SEQ ID NO:174:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:

CTACCTACGA TCTGACTAGC CACGATAATC CTCCGAAAGC GTTGTCCGAA 50
TGGGTCGTTA GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:175:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:

ATCCGCCTGA TTAGCGATAC TTATCACCCC CACTGGATAG AGCCGCAGCG 50
TGCCCCTACT ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:176:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:

ATCCGCCTGA TTAGCGATAC TGCCCACTGC ATAGAGGGAC GGTTGTTTCC 50
GCCCCTGTT TACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:177:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:177:

CTACCTACGA TCTGACTAGC GTGAAGGAGC CCCAACTGGA TAGAAGCCTT 50
AAGGCGGTGT GCTTACTCTC ATGTAGTTCC T 81

(2) INFORMATION FOR SEQ ID NO:178:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:178:
CTACCTACGA TCTGACTAGC CCACCGCAGA GTGTTACACC CCATAGGAGA 50
AGTCCGGATG GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:179:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 87 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:179:
CTACCTACGA TCTGACTAGC CCACTGCATA GAGAGTCGCA AGACACGGTG 50
CTTTATTCNC CGCTTAGCTT ACTCTCATGT AGTTCCT 87

(2) INFORMATION FOR SEQ ID NO:180:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:180:
CTACCTACGA TCTGACTAGC TGCCCCACTG GATAGAGTAG GAGGCCTAGC 50
CGACACGGTG CTTAGCTTAC TCTCATGTAG TTCCT 85

(2) INFORMATION FOR SEQ ID NO:181:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:181:
CTACCTACGA TCTGACTAGC CGAGGTCCCC CACTGGATAG AGTTGTTGAA 50
ACAACGGTGC GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:182:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:182:
CTACCTACGA TCTGACTAGC AACACTTCCC CACTGGATAG AGGCCTTTCG 50
CAGAGCCGGT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:183:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:183:

CTACCTACGA TCTGACTAGC CCACTGCATA GAGAACTGGA TCGACGGTCC	50
AAAGTTCGGT GCTTAGCTTA CTCTCATGTA GTTCCT	86

(2) INFORMATION FOR SEQ ID NO:184:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 89 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:184:

CTACCTACGA TCTGACTAGC CCACTGCATA GAGATACTGG ATTCGACNNN	50
CCAAAGTTTC GGTGCTTAGC TTA CTCTCAT GTAGTTCCT	89

(2) INFORMATION FOR SEQ ID NO:185:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:185:

CTACCTACGA TCTGACTAGC CCACTGCAGA GAGTCAACCT TACGANGCCA	50
AGGTTGCGGT GCTTAGCTTA CTCTCATGTA GTTCCT	86

(2) INFORMATION FOR SEQ ID NO:186:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:186:

ATCCGCCTGA TTAGCGATAC TTCTGCGAGA GACCTACTGG AACGTTTGT	50
GATATTCACA ACTTGAGCAA AATCACCTGC AGGGG	85

(2) INFORMATION FOR SEQ ID NO:187:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:187:
 ATCCGCCTGA TTAGCGATAC TATACACCCG GCGGGCCTAC CGGATCGTTG 50
 ATTTCTCTCC ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:188:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:188:
 ATCCGCCTGA TTAGCGATAC TACGCCCCCT GAGACCTACC GGAATNTTNT 50
 CGCTAGGCCT AACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:189:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:
 ATCCGCCTGA TTAGCGATAC TGGGCATCTA ACCCAGACCT ACCGGAACGT 50
 TATCGCTTGT GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:190:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 83 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:
 ATCCGCCTGA TTAGCGATAC TGGTGTGAAC CAGACCTACN GGAACGTTAT 50
 CGCTTGTGAC TTGAGCAAAA TCACCTGCAG GGG 83

(2) INFORMATION FOR SEQ ID NO:191:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:
 ATCCGCCTGA TTAGCGATAC TCATCAGTAT TATATAACGG GAACCAACGG 50
 CAAATGCTGA CACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:192:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 85 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:
ATCCGCCTGA TTAGCGATAC TTCCNNGGGA GAATAGGGTT AGTCGGAGAA 50
GTTAATCGCT ACTTGAGCAA AATCACCTGC AGGGG 85
- (2) INFORMATION FOR SEQ ID NO:193:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 86 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:
ATCCGCCTGA TTAGCGATAC TCGGGAACGT GTGGTTACNC GGCCTACTGG 50
ATTGTTTCCT GACTTGAGCA AAATCACCTG CAGGGG 86
- (2) INFORMATION FOR SEQ ID NO:194:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 81 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:
ATCCGCCTGA TTAGCGATAC TGGTAGGTCC GGTGTGAAAG AGGTTCGCAT 50
CAGGTAACCT GAGCAAAATC ACCTGCAGGG G 81
- (2) INFORMATION FOR SEQ ID NO:195:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 85 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:
ATCCGCCTGA TTAGCGATAC TCCTCAGGCA ACATAGTTGA GCATCGTATC 50
GATCCTGGAG ACTTGAGCAA AATCACCTGC AGGGG 85
- (2) INFORMATION FOR SEQ ID NO:196:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 84 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:
ATCCGCCTGA TTAGCGATAC TTTGGCTTGA GTCCCGGGAC GCACTGTTGA 50

CAGTGGAGTA CTTGAGCAAA ATCACCTGCA GGGG

84

(2) INFORMATION FOR SEQ ID NO:197:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:

ATCCGCCTGA TTAGCGATAC TCAGCAGGTT AGTATAACGG GAACCAACGG
CAAATGCTGA CACTTGAGCA AAATCACCTG CAGGGG

50

86

(2) INFORMATION FOR SEQ ID NO:198:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:

CTACCTACGA TCTGACTAGC GCAAGGGCAT CTCGGAATCG GTTAATCTGA
CTTGCAATAC GCTTAGCTTA CTCTCATGTA GTTCCT

50

86

(2) INFORMATION FOR SEQ ID NO:199:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:

CTACCTACGA TCTGACTAGC GATCCACGAA GAAGCTTACT CTCATGTAGT
TCCAGCTTAC TCTCATGTAG TTCCT

50

75

(2) INFORMATION FOR SEQ ID NO:200:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:

GTACCATCCA CGGTTTACGT GGACAAGAGG GCCCTGGTAC

40

(2) INFORMATION FOR SEQ ID NO:201:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:
GTAGTTCCTT TAGGACTAGA GGGCCGCCTA C 31
- (2) INFORMATION FOR SEQ ID NO:202:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:
TGGACTAGAG GNCAGCAAAC GATCCTTGGT TCGCGTCC 38
- (2) INFORMATION FOR SEQ ID NO:203:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:
CCCGTCTTCC AGACAAGAGT GCAGGG 26
- (2) INFORMATION FOR SEQ ID NO:204:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:
AGGGCCACGT CTATTTAGAC TAGAGTGCAG TGGTTC 36
- (2) INFORMATION FOR SEQ ID NO:205:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:
GGAGGTGCGT TGTCCGAAGG GGTCGTTAGT CACCTC 36
- (2) INFORMATION FOR SEQ ID NO:206:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:
GCAAGGGGTC CTGCCGAATG GGGGAATACG CTAGCCGAAA 40
- (2) INFORMATION FOR SEQ ID NO:207:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:207:
CGAGGTCCCC CACTGGATAG AGTTGTTGAA ACAACGGTGC GCTTA 45
- (2) INFORMATION FOR SEQ ID NO:208:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:
AACACTTCCC CACTGGATAG AGGCCTTTCG CAGAGCCGGT GCTTA 45
- (2) INFORMATION FOR SEQ ID NO:209:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:
GGGCATCTAA CCCAGACCTA CCGGAACGTT ATCGCTTGTG 40
- (2) INFORMATION FOR SEQ ID NO:210:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:
AGACAAGAGT GCAGG 15
- (2) INFORMATION FOR SEQ ID NO:211:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:

GGACTAGAGG GCAGT

15

(2) INFORMATION FOR SEQ ID NO:212:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:

CTCCGAATGG GGNAAAG

18

(2) INFORMATION FOR SEQ ID NO:213:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:

CCCCACTGGA TAGAG

15

(2) INFORMATION FOR SEQ ID NO:214:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:

CCCCACTGCA TAGAG

15

(2) INFORMATION FOR SEQ ID NO:215:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215:

AGACCTACCG GAACGTT

17

(2) INFORMATION FOR SEQ ID NO:216:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 86 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216:

ATCCGCCTGA TTAGCGATAC TNNNNNNNNNN NNNNNNNNNN NNNNNNNNNN

50

NNNNNNNNNN NACTTGAGCA AAATCACCTG CAGGGG

86

(2) INFORMATION FOR SEQ ID NO:217:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 1
- (D) OTHER INFORMATION: N equals 3 biotin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:217:

NCCCCTGCAG GTGATTTTGC TCAAGT

26

(2) INFORMATION FOR SEQ ID NO:218:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:218:

ATCCGCCTGA TTAGCGATAC T

21

(2) INFORMATION FOR SEQ ID NO:219:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:219:

CTACCTACGA TCTGACTAGC NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
NNNNNNNNNN GCTTACTCTC ATGTAGTTCC T

50

81

(2) INFORMATION FOR SEQ ID NO:220:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: modified base
- (B) LOCATION: 2 and 4
- (D) OTHER INFORMATION: N is biotin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220:

ANANAGGAAC TACATGAGAG TAAGC

25

(2) INFORMATION FOR SEQ ID NO:221:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221:

CTACCTACGA TCTGACTAGC

20

(2) INFORMATION FOR SEQ ID NO:222:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:222:

GGGAGACAAG AATAAACGCT CAANNNNNNN NNNNNNNNNN NNNNNNNNNN

50

NNNNNNNNNN NNNNNNNNNN NNNTTCGACA GGAGGCTCAC AACAGGC

97

(2) INFORMATION FOR SEQ ID NO:223:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:

TAATACGACT CACTATAGGG AGACAAGAAT AAACGCTCAA

40

(2) INFORMATION FOR SEQ ID NO:224:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:224:

GCCTGTTGTG AGCCTCCTGT CGAA

24

(2) INFORMATION FOR SEQ ID NO:225:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:225:

GGGAGACAAG AAUAAACGCU CAANNNNNNN NNNNNNNNNN NNNNNNNNNN	50
NNNNNNNNNN NNNNNNNNNN NNNUUCGACA GGAGGCUCAC AACAGGC	97

(2) INFORMATION FOR SEQ ID NO:226:

- (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:226:

GCCGGATCCG CCTGTTGTGA GCCTCCTGTC GAA	33
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(2) INFORMATION FOR SEQ ID NO:227:

- (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 48 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:227:

CCGAAGCTTA ATACGACTCA CTATAGGGAG ACAAGAATAA ACGCTCAA	48
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(2) INFORMATION FOR SEQ ID NO:228:

- (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:228:

TAATACGACT CACTATA	17
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(2) INFORMATION FOR SEQ ID NO:229:

- (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 97 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:229:
GGGAGACAAG AAUAAACGCU CAAUUGUGAU CUACCGUAC CUGACGGACG 50
UGUUUUUACA CCAACGAACC UGGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:230:

- (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 97 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:230:
GGGAGACAAG AAUAAACGCU CAAUGAACCU CUGAGUUCUU CAUAGAACUG 50
AUAUCUCAA GAAGGGGUAG AUGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:231:

- (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 97 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:231:
GGGAGACAAG AAUAAACGCU CAACGUCUUC UACAGGGAAC CGUGGUGCAU 50
CUGUGAAGUU GUAGAUUCCU AGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:232:

- (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 97 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:232:
GGGAGACAAG AAUAAACGCU CAAGGGUUGA UUACCAAUUC GUAACCUGUA 50
CCCUGCCUAC ACUAGGACAA CGGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:233:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:233:

GGGAGACAAG AAUAAACGCU CAACUAACCG AAGCUCUGAG AAUAGUUUA 50
UCCAGUGAAU GAAUCCUGAU GGGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:234:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:234:

GGGAGACAAG AAUAAACGCU CAACGAGCUU UUAGUAAGAC UCAUGCCGAG 50
AAAUCCGGUCG UGAUGCUGUC GAGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:235:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:235:

GGGAGACAAG AAUAAACGCU CAAGCCCGGG AAUUGCAUGU UGUGCGUGCC 50
GGGAGUCCAA GUCAGCAUCC UCAUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:236:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:236:

GGGAGACAAG AAUAAACGCU CAAGGGUUGA UUACCAAUC GUAACCUGUA 50
CCCUGCCUAC CCUAGGACAA CGAUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:237:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:237:

GGGAGACAGA AUAAACGCUC AAUCCCCACU CGGUCGUGAC CUGACGUGAA 50
AACGGAUAGG AUCGCACGUC AAUUCGACAG GAGGCUCACA ACAGGC 96

(2) INFORMATION FOR SEQ ID NO:238:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:238:

GGGAGACAAG AAUAAACGCU CAAGUGUCCC UUGUCACCUG GGACUGGGCC 50
GUUGAACUG ACAUUCUAUA CGAUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:239:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:239:

GGGAGACAAG AAUAAACGCU CAAGGCCAAC GUCCGAGUUG GUUCCCCAUA 50
GCUGAGCACA GGACGGCUUC UGCUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:240:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:240:

GGGAGACAAG AAUAAACGCU CAACCCGGAU GGUGAGCCCU GUCCGAGAUU 50

GGCACGAGGG UAAAGGGUAG GGAUUCGACA GGAGGCUCAC AACAGGC

97

(2) INFORMATION FOR SEQ ID NO:241:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:241:

GGGAGACAAG AAUAAACGCU CAACUGUUGC UAAGUAGAAG UCAUAUUCUG

50

CGAUGGUUAA AGAUAACCCA GCCUUCGACA GGAGGCUCAC AACAGGC

97

(2) INFORMATION FOR SEQ ID NO:242:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 95 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:242:

GGGAGACAAG AAUAAACGCU CAAUCUCGUA UCAGCACACC GGUACAAAGA

50

GGGAUGCAAU NCGCCUGUGA CUUCGACAGG AGGCUCACAA CAGGC

95

(2) INFORMATION FOR SEQ ID NO:243:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 100 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:243:

GGGAGACAAG AAUAAACGCU CAACGUCUUC UACAGGGAAC CGUGGUGCAU

50

CUGUGAAGUU GUUGUAGAUU CCUAGAUUCG ACAGGAGGCU CACAACAGGC

100

(2) INFORMATION FOR SEQ ID NO:244:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:244:

GGGAGACAAG AAUAAACGCU CAAUGUCUUG GCGUCCACGU CGUAGUGUGU 50
GGGGGGAAAA GAGGAGGGUG CACUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:245:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:245:

GGGAGACAAG AAUAAACGCU CAAUGUUGAG CCUGGUGAAG CAUGUACUGC 50
GAAGUGGAGG GAAUGUGGAA GGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:246:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:246:

GGGAGACAAG AAUAAACGCU CAAGCUCCCC UAGCCGACUU CCUGUUGGAC 50
GAGGGUGGUA CCGUGGGAGG AUGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:247:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:247:

GGGAGACAAG AAUAAACGCU CAACGAGCCU UUAGUAAGAC UCAUGCCGAG 50
AAAUCCGUUC UGAUGCUGUC GAGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:248:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:248:

GGGAGACAAG AAUAAACGCU CAAUUCGUGA GGGCGGUGUG GGAGGCAAGC 50
GGUACGAGCG UACUGUCUGG GCCUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:249:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:249:

GGGAAAAGCG AATCATACAC AAGANNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN NNNNNNNNNN NNNNGCTCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:250:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:250:

TAATACGACT CACTATAGGG AAAAGCGAAT CATACACAAG A 41

(2) INFORMATION FOR SEQ ID NO:251:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:251:

TTCTCGGTTG GTCTCTGGCG GAGC 24

(2) INFORMATION FOR SEQ ID NO:252:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:252:

GGGAAAAGCG AAUCAUACAC AAGANNNNNN NNNNNNNNNN NNNNNNNNNN	50
NNNNNNNNNN NNNNNNNNNN NNNNGCUCCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:253:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:253:

CGCGGATCCT AATACGACTC ACTATAGGGA AAAGCGAATC ATACACAAGA	50
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(2) INFORMATION FOR SEQ ID NO:254:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:254:

GGCGAATTCT TCTCGTTGG TCTCTGGCGG AGC	33
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(2) INFORMATION FOR SEQ ID NO:255:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:255:

GGGAAAAGCG AAUCAUACAC AAGAUGUGAU GGGUGUGUUU UGGUUAGCUG	50
UGGAGGGCCA UGUGGGCUGG ACGGCUCCGC CAGAGACCAA CCGAGAA	97

(2) INFORMATION FOR SEQ ID NO:256:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:256:

GGGAAAAGCG AAUCAUACAC AAGAGGUGUG UGUGCGCCGC GAGGUGUCUG	50
AGGAGAGAGG UGGCAGUGGA GGGUGCUC CGAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:257:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:257:

GGGAAAAGCG AAUCAUACAC AAGAGGAGGG UGUGCGGACG GGAGCGUGUA	50
GUGAGGCUUU UCAGGCGUUG GACGGCUC CGAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:258:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:258:

GGGAAAAGCG AAUCAUACAC AAGAUGUGAU GGGUGUGAUU UUGGUUAGCU	50
GUGGAGGGUU AUGUGGGUCG GACGGCUC CGAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:259:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:259:

GGGAAAAGCG AAUCAUACAC AAGAUGUGG CUUCCUGAGG GGUAGGAACU	50
CUGAAGUCAU GGUUCGUGGU AAGCGCUC CGAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:260:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:260:
GGGAAAAGCG AAUCAUACAC AAGAGGAGGG AAUGUGGAAG GGUUGUGGU 50
GUUUCGCAAA UGCCGCAUGG ACGUGCUC CGAGAGACCA ACCGAGAA 98

- (2) INFORMATION FOR SEQ ID NO:261:
 - (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 98 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:261:
GGGAAAAGCG AAUCAUACAC AAGAGGGGGG AGAGGCGUGG ACGAUGUUGU 50
GGUUAUGCUG UCGGUUUUGG CUUGGCUC CGAGAGACCA ACCGAGAA 98

- (2) INFORMATION FOR SEQ ID NO:262:
 - (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 98 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:262:
GGGAAAAGCG AAUCAUACAC AAGAGGGAUG GAUCGGUGAG ACGAGCAGUG 50
GAGUGGUGAG GUGUGGUGUC ACGUGCUC CGAGAGACCA ACCGAGAA 98

- (2) INFORMATION FOR SEQ ID NO:263:
 - (i) SEQUENCE CHARACTERIZATION:
 - (A) LENGTH: 98 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:263:
GGGAAAAGCG AAUCAUACAC AAGACGUUCG GUGGUGGACA GGGUAAUGUG 50
GAGGGACCGG GUGAUUGUGU AUGUGCUC CGAGAGACCA ACCGAGAA 98

- (2) INFORMATION FOR SEQ ID NO:264:
 - (i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:264:

GGGAAAAGCG AAUCAUACAC AAGAUGGAGG GUGGCGGGAG AAGUUUGAGG	50
UCGGGGUGCU AUGAUGUGCG CUAGGCUCCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:265:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:265:

GGGAAAAGCG AAUCAUACAC AAGAUACGA CGGAGGGCGG AUGAAGGGGG	50
GAAGGUCGUG AGUCCAUGCC GUGUGCUCCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:266:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:266:

GGGAAAAGCG AAUCAUACAC AAGAGGAUGG GCGUGAAUGG AGGGCAAUGU	50
GUGGGUUUGU UGAGCCAGUU GGUGCUCGCG CAGAGACCAA CCGAGAA	97

(2) INFORMATION FOR SEQ ID NO:267:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:267:

GGGAAAAGCG AAUCAUACAC AAGAACGGGU CCCUAGACG UCGUUUCGAG	50
AUGUGUGUUU CGGAAAGAGU AGCGGCUCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:268:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:268:

GGGAAAAGCG AAUCAUACAC AAGAACAGCG AUUUGUGUGC UCGUCGGCUG	50
UUUGCAGAUG UAUGGAACCA UGCAGCUCCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:269:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:269:

GGGAAAAGCG AAUCAUACAC AAGAGGGCUU GGGGUGGGGU CUUGCAGACG	50
GAUAUGGUAA GCUGCUGCUG GCUGCUCGCG CAGAGACCAA CCGAGAA	97

(2) INFORMATION FOR SEQ ID NO:270:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:270:

GGGAAAAGCG AAUCAUACAC AAGAAUUGGG AACGUGGCGC AUCGGGAGUG	50
UAUGGUGGCU UGUGGGCGGG UUAGCUCGCG CAGAGACCAA CCGAGAA	97

(2) INFORMATION FOR SEQ ID NO:271:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:271:

GGGAAAAGCG AAUCAUACAC AAGAAGCCAG GUCUCAAUG GUAGUUGUUG	50
AUUGGUGGUG CUUUGCAGAU GUAUGCUCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:272:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:272:

GGGAAAAGCG AAUCAUACAC AAGAUCGGUU AUGGAGAGGC GGUAGAGAGC	50
GAGUAGCAAG UAACGGUGCA AAAGGCUCCG CCAGAGACCA ACCGAGAA	98

(2) INFORMATION FOR SEQ ID NO:273:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 99 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:273:

GGGAAAAGCG AAUCAUACAC AAGACCGCAC UUCGAUUGCA UUGUCGAGGA	50
GAGCGGGUGC AGAUGUACGG GUUUGGCUCG GCCAGAGACC AACCGAGAA	99

(2) INFORMATION FOR SEQ ID NO:274:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 99 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:274:

GGGAAAAGCG AAUCAUACAC AAGAAGACUU GGUACGCGUG GUUAGCUAGG	50
AGGGGGGGGAG AAGAUAGGC GAUAGGCUCG GCCAGAGACC AACCGAGAA	99

(2) INFORMATION FOR SEQ ID NO:275:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:275:

GGGAAAAGCG AAUCAUACAC AAGAGAGUUU UGCUUCGACA UGGGGUUAGC	50
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UGUGGAGCAG GGUGACCUGG GACCGCUCCG CCAGAGACCA ACCGAGAA

98

(2) INFORMATION FOR SEQ ID NO:276:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 98 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:276:

GGGAAAAGCG AAUCAUACAC AAGAUGGAGU GGGUAGGCGU GGGAGGUAUG

50

GCAUCUUGUG UGUGCCAAGA UGGUGCUCCG CCAGAGACCA ACCGAGAA

98

(2) INFORMATION FOR SEQ ID NO:277:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:277:

GGGUCAUACA CAAGAACAGU GAUUUGUGUG CUCGUCGGCU GUUUGCGAGU

50

UAU

53

(2) INFORMATION FOR SEQ ID NO:278:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 63 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:278:

GGGAUACACA AGAAGCCAGG UCUUCAUUGG UAGUUGUUGA UUGGUGGUGC

50

UUUGCAGAUU UAU

63

(2) INFORMATION FOR SEQ ID NO:279:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:279:
GGGAGAGGCG GUAGAGAGCG AGUAGCAAGU AACGGUGCAA AAGGCUCCGC 50
C 51

- (2) INFORMATION FOR SEQ ID NO:280:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:280:
GGGUUUGGUU AGCUGUGGAG GGCCAUGAGG GCUGGA 36

- (2) INFORMATION FOR SEQ ID NO:281:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 65 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:281:
GGGCAUACAC AAGACGUUCG GUGGUGGACA GGUAAUGUG GAGGGACCGG 50
GUGAUUGUGU AUGUG 65

- (2) INFORMATION FOR SEQ ID NO:282:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 58 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:282:
GGAUCACGAC GGAGGGCGGA UGAAGGGGGG AAGGUCGUGA GUCCAUGCCG 50
UGUGCUC 58

- (2) INFORMATION FOR SEQ ID NO:283:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:283:
GGGCGUGGUG CAUCUGUGCC C 21
- (2) INFORMATION FOR SEQ ID NO:284:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:284:
GGGAGCUACA GGGAACCGUG GUGCAUCUG 29
- (2) INFORMATION FOR SEQ ID NO:285:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:285:
GGGGUAUCAG CACACCGGUA CAAAGAGGAU GC 32
- (2) INFORMATION FOR SEQ ID NO:286:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:286:
GGGAAAAGCG AAUCAUACAC AAGAUCCGUU AUGGAGAGGC GGUAGAGAGC 50
GAGUAAAGUA ACGGUGCAAA AGGCUCCGCC AGAGACCAAC CGAGAA 96
- (2) INFORMATION FOR SEQ ID NO:287:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:287:

GGGAGACAAG AAUAAACGCU CAACGUCUUC UACAGGGAAC CGUGGUCCAU 50
CUGUGAAGUU GUAGAUUCCU AGUUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:288:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 97 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:288:

GGGAGACAAG AAUAAACGCU CAACGUCUUC UACAGGGAAC CGUGGCGCGC 50
CUGUGAAGUU GUAGAUUCCU AGUUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:289:

(i) SEQUENCE CHARACTERIZATION:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:289:

RNYAGNNNNN NRGNRNCANR NRRNGNUGNA 30

We Claim:

1. A method for identifying nucleic acid ligands and nucleic acid ligand sequences to secretory phospholipase A₂ (sPLA₂) comprising:
 - a) contacting a candidate mixture of nucleic acids with sPLA₂, wherein nucleic acids having an increased affinity to the sPLA₂ relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;
 - b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
 - c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to sPLA₂, whereby nucleic acid ligands of sPLA₂ may be identified.
2. The method of Claim 1 further comprising:
 - d) repeating steps a), b) and c).
3. The method of Claim 1 wherein said candidate mixture is comprised of single-stranded nucleic acids.
4. The method of Claim 3 wherein said single-stranded nucleic acids are ribonucleic acids.
5. The method of Claim 3 wherein said single-stranded nucleic acids are deoxyribonucleic acids.
6. A nucleic acid ligand to sPLA₂ identified according to the method of Claim 1.
7. A purified and isolated non-naturally occurring nucleic acid ligand to secretory phospholipase A₂ (sPLA₂).

8. The nucleic acid ligand of Claim 7 which is a ribonucleic acid ligand.
9. The RNA ligand of Claim 8 wherein said RNA ligand is selected from the group consisting of the nucleotide sequences set forth in Table 2.
10. The RNA ligand of Claim 9 wherein said ligand is selected from the group consisting of SEQ ID NOS: 10-122.
11. A purified and isolated non-naturally occurring RNA ligand to sPLA₂, wherein said ligand is substantially homologous to and has substantially the same ability to bind sPLA₂ as a ligand selected from the group consisting of the sequences set forth in Table 2.
12. A purified and isolated non-naturally occurring RNA ligand to sPLA₂, wherein said ligand has substantially the same structure and the same ability to bind sPLA₂ as a ligand selected from the group consisting of the sequences set forth in Table 2.
13. A purified and isolated non-naturally occurring RNA ligand to sPLA₂ comprising the sequences selected from the group consisting of SEQ ID NOS: 97-100.
14. The ligand of Claim 7 wherein said ligand has been chemically modified at the sugar and/or phosphate and/or base.
15. A purified and isolated non-naturally occurring nucleic acid ligand to VEGF, wherein said nucleic acid ligand is deoxyribonucleic acid.
16. The DNA ligand of Claim 15 wherein said ligand is selected from the group consisting of the sequences set forth in Table 11.

17. The DNA ligand of Claim 15 wherein said ligand is substantially homologous to and has substantially the same ability to bind VEGF as a ligand selected from the group consisting of the sequences set forth in Table 11.

18. The DNA ligand of Claim 15 wherein said ligand has substantially the same structure and substantially the same ability to bind VEGF as a ligand selected from the group consisting of the sequences set forth in Table 11.

19. A purified and isolated non-naturally occurring nucleic acid ligand to VEGF comprising modified nucleic acids.

20. The nucleic acid ligand of claim 19 wherein said modified nucleic acid is selected from the group consisting of 2'-NH₂ nucleosides, 2'-OMe nucleosides, and phosphorothioate nucleotides.

21. The nucleic acid ligand of claim 20 comprising multiple modified nucleic acids.

22. The nucleic acid ligand of claim 21 wherein said nucleic acid ligand is selected from the group consisting of the sequences set forth in Table 7.

23. A method of identifying nucleic acid ligands and nucleic acid ligand sequences to HIV-1 GAG, comprising:

a) contacting the candidate mixture of nucleic acids with HIV-1 GAG, wherein nucleic acids having an increased affinity to HIV-1 GAG relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to HIV-1 GAG, whereby nucleic acid ligands of HIV-1 GAG may be identified.

24. The method of claim 1 further comprising:

d) repeating steps a), b), and c).

25. The method of claim 23 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.

26. The method of claim 25 wherein said single stranded nucleic acids are ribonucleic acids.

27. The method of claim 26 wherein said nucleic acids are modified nucleic acids.

28. The method of claim 27 wherein said nucleic acids are 2'-amino (2'-NH₂) modified ribonucleic acids.

29. The method of claim 27 wherein said nucleic acids are 2'-fluoro (2'-F) modified ribonucleic acids.

30. A nucleic acid ligand to HIV-1 GAG identified according to the method of claim 23.

31. A purified and isolated non-naturally occurring RNA ligand to HIV-1 GAG wherein said ligand is selected from the group consisting of the sequences set forth in Tables 13, 15, 18, 19, and 20.

32. A purified and isolated non-naturally occurring RNA ligand to HIV-1 GAG wherein said ligand is substantially homologous to and has substantially the same ability to bind HIV-1 GAG as a ligand selected from the group consisting of the sequences set forth in Tables 13, 15, 18, 19, and 20.

33. A purified and isolated non-naturally occurring RNA ligand to HIV-1 GAG wherein said ligand has substantially the same structure and substantially the same ability to bind HIV-1 GAG as a ligand selected from the group consisting of the sequences set forth in Tables 13, 15, 18, 19, and 20.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02942**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C07H 21/02, 21/04; C12P 19/34; C12Q 1/68

US CL : 435/6, 91.2; 536/22.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/22.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
935/77, 78

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,270,163 A (L. GOLD ET AL.) 14 December 1993, column 5, lines 32-49; Figure 2; columns 44-45, Example 4.	1-8, 11, 12, 14
Y	WO 91/19813 A1 (THE UNIVERSITY OF COLORADO FOUNDATION, INC.) 26 December 1991, page 9, lines 13-32; Figure 2; pages 82-84, Example 4.	1-8, 11, 12, 14
Y	KRAMER et al. Structure and properties of a human non-pancreatic phospholipase A2. The Journal of Biological Chemistry. 05 April 1989, Vol. 264, No. 10, pages 5768-5775, especially page 5768, paragraph 2.	1-8, 11, 12, 14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

A	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	*I*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E	earlier document published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, use, exhibition or other means	*Z*	document member of the same patent family
P	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 JUNE 1996

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02942

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-14, drawn to a first process of identifying nucleic acid ligands to sPLA2 and a first product, nucleic acid ligands thereby identified;

Group II, claim(s) 15-22, drawn to a second product, nucleic acid ligands to VEGF;

Group III, claim(s) 23-29, drawn to a second process of identifying nucleic acid ligands to HIV-1 GAG;

Group IV, claims 30-33, drawn to a third product, nucleic acids to HIV-1 GAG.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the first and second processes differ in the target for which the ligands are identified and the first, second and third products are ligands to distinct targets.